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THE ESTIMATION AND CHARACTERIZATION OF PLANKTON POPULATIONS BY PIGMENT ANALYSIS. III. A NOTE ON THE USE OF "MILLIPORE" MEMBRANE FILTERS IN THE ESTIMATION OF PLANKTON PIGMENTS.[1,2]

By

GRACE I. CREITZ and FRANCIS A. RICHARDS

Woods Hole Oceanographic Institution

ABSTRACT

Collection of plankton samples on "AA" (aerosol assay) type "Millipore" membrane filters has been substituted for centrifugation in the method of Richards with Thompson for estimating plankton pigments. Although the filter dissolves in the 90% acetone that is subsequently used for extracting the pigments, its solution does not interfere with the spectrophotometric determination of the chlorophyll and carotenoid components. Comparison between retention by Millipore filters and the Foerst plankton centrifuge indicates that the filters are significantly more efficient. Plankton samples can be stored dry, in the dark, under vacuum, for at least three weeks without affecting the pigments for subsequent analysis.

During the work reported in the second paper of this series (5), various methods for removing plankton from water were investigated, and the Foerst plankton centrifuge was selected as the most satisfactory. Since that time, Millipore membrane filters[3] have become

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[2] Part of this work has been supported by a grant from the National Science Foundation. This financial aid is gratefully acknowledged.

[3] Manufactured under the name "Millipore" by the Lovell Chemical Company, Watertown, Massachusetts (4); available in two grades, designated "HA" (hydrosol assay) and "AA" (aerosol assay).

readily available and the procedures previously described have been varied to incorporate their use.

The method, as revised, is as follows: filter an adequate quantity of water containing about 0.1 g of powdered magnesium carbonate through an AA Millipore filter, trim the filter to the size of its effective filtering area, fold and roll it into a cylinder and drop it into a 16 × 125 mm screw-cap test tube. Place the tube in a vacuum desiccator to be dried and stored if immediate extraction is impractical. Preliminary investigations indicate that dried plankton samples may be stored at least three weeks without apparent change in the absorption spectra of their extracts by refrigerating the samples in a vacuum desiccator. This procedure protects them from oxygen, moisture, light and high temperatures. Extraction and estimation are carried out according to the procedures of Richards with Thompson (5).

Membrane filters have been described by Goetz and Tsuneishi (2) and by Bush (1), and their use in plankton collection has been described by Goldberg, Baker and Fox (3).

Despite their designation as AA filters, they have proved applicable for use with aqueous suspensions. According to a personal communication from W. B. Krabek of the Lovell Chemical Company, a suspension of *Serratia marcescens* (coccoid, 0.5 μ, or short rods, 0.5 × 0.5 − 1.0 μ) containing 8×10^6 organisms was almost completely retained by AA filters, 25 or fewer colonies appearing in the filtrate. In this laboratory, aerosol filters have retained completely the cells of a *Nannochloris* species culture (1.0 − 2.5 μ). These observations as well as those on the retention of a large number of natural plankton populations lead to the conclusion that AA filters will retain all natural plankton organisms down to 1 μ in diameter. The HA filter, though it has a somewhat smaller pore size, filters less freely, is more brittle and bulky, and has a somewhat greater absorbancy when placed in 90% acetone solution (Table I); therefore, the AA filter is now used exclusively in this laboratory.

Table I. Absorbancies (log I_0/I) of solutions of Millipore filters in five ml of 90% acetone, in absorption cells of 5 cm light path.

Wavelength $m\mu$	*AA Type (Entire, 4.7 cm. diam.)*	*AA Type (Trimmed to ca. 3.9 cm diam.)*	*HA Type (Entire, 4.7 cm diam.)*
480	.005	.002	.012
510	.004	.001	.010
630	.002	.000	.006
645	.001	.000	.006
665	.001	.000	.005
750	.001	.000	.004

The volume to be filtered varies with the type of water under investigation. The filters act as two-dimensional screens, permitting water to pass through them rapidly until all pores are covered; after that time, additional water can be filtered only very slowly. The amount of water which can be filtered in a reasonable time depends on the amount and kind of particulate matter present. Small volumes can be filtered when the plankton crop is large, large volumes when the crop is small. Nonpigmented detritus can also clog the filters, and in extreme cases sufficient amounts of plankton for a satisfactory analysis might not be collected. The volumes of natural waters which have been used in this laboratory have varied from a few hundred milliliters to more than six liters. Much smaller volumes of algal cultures have been used. To insure representative sampling, precautions must be taken against the settling onto the membrane of particulate matter from water which will not be filtered.

Absorbancy of Millipore Filters. Millipore filters are almost completely soluble in 90% acetone. Clear solutions are obtained on centrifuging for about 5 minutes in a clinical centrifuge in the presence of magnesium carbonate. Solutions of AA Millipore filters have negligible absorbancies at all wavelengths that are used to compute pigment concentrations (Table I). The absorbancy is decreased when the filters are trimmed to remove the unexposed edge; this removes about 30% of the total bulk of the filter.

Millipore filters were added to a large number of solutions containing plankton pigments, and the resultant spectra were compared with those of the original pigment solutions. Examples of two such experiments are shown in Table II. There is no evidence of chemical interaction between the filter and the pigments to create discrepancies in the absorption spectra. The small effects which the filters have on the pigment spectra are similar to Rayleigh scattering and probably result from incomplete solution of the filter and (or) gel-stabilized colloidal suspensions of particulate matter. In these studies, representing a variety of concentrations, average deviation of chlorophyll *a* estimates, either with or without added filters, was less than 5%.

Scattering can be detected from the absorbancy at 750 mμ, where pigment absorbancies are nearly negligible. If the absorbancy at this wavelength is appreciable, further centrifugation in the presence of magnesium carbonate is probably necessary. Even slight turbidities cause erroneously high estimates of components other than chlorophyll *a*. The chlorophyll *a* estimates are not as greatly affected but tend to be slightly low.

Table II. Absorbancies (log I_0/I) in 5 cm absorption cells, computed pigment concentrations of plankton extracts with and without added Millipore (AA) filter.

	ABSORBANCY			
	Station 11		*Station 12*	
Wavelength, mμ.	*Without M.F. added*	*With M.F. added*	*Without M.F. added*	*With M.F. added*
480	1.020	1.015	.455	.468
510	.390	.395	.185	.202
630	.197	.197	.076	.075
645	.220	.220	.089	.088
665	.790	.795	.239	.238
750	.009	.015	.009	.012
	COMPUTED CONCENTRATIONS			
Chlorophyll *a*,mg/l	11.72	11.80	3.49	3.48
Chlorophyll *b*,mg/l	0.05	0.02	0.43	0.42
Chlorophyll *c*,MSPU/L	5.28	5.23	2.75	2.70
Non-astacin carotenoids, MSPU/L	3.41	3.56	1.33	1.24
Astacin-type carotenoids, MSPU/L	1.16	1.21	0.75	0.78

Comparisons of Retention by the Foerst Plankton Centrifuge and Millipore Filters. It is well known that the recovery of plankton organisms by the continuous-feeding plankton centrifuge depends on several factors, including the speed of the instrument, the size and relative density of the plankton organisms, and the rate of flow through the centrifuge. The membrane filters provide a good means for investigating the retention by the centrifuge.

Aliquot samples of sea water were passed through AA Millipore filters and through the Foerst plankton centrifuge at a rate of about seven minutes per liter. The material passed by the centrifuge was subsequently filtered. The differences in retention by the two methods were large, the chlorophyll *a* retained by the centrifuge varying between 69 and 86% of that retained by the filters. The sum of the pigment contents of the material retained and passed by the centrifuge agreed well with the pigments retained by filtration.

In the examples in Table III, the material retained by the centrifuge was comparatively poor in chlorophyll *c* whereas the material recovered on filtration of the centrifugate was rich in this compound.

Table III. Comparison of retention by Foerst plankton centrifuge and AA type Millipore filters.

Sample			*Chlorophyll a* mg/M^3	*Chlorophyll b* mg/M^3	*Chlorophyll c* $MSPU/M^3$		$\frac{Chph\ b}{Chph\ a}$	$\frac{Chph\ c}{Chph\ a}$
21	Retained by Centrifuge		2.52	0.76	3.33		0.30	1.32
	Passed by Centrifuge		1.04	0.48	2.17		0.46	2.05
		Sum	3.56	1.24	5.50	$\Sigma/\Sigma Ca$	0.35	1.54
	Retained by M.F.		3.58	1.05	5.80		0.29	1.62
15	Retained by Centrifuge		4.68	0.76	4.83		0.16	1.03
	Passed by Centrifuge		0.97	0.48	2.65		0.50	2.74
		Sum	5.65	1.24	7.48	$\Sigma/\Sigma Ca$	0.22	1.32
	Retained by M.F.		5.94	1.40	8.00		0.24	1.35
8	Retained by Centrifuge		8.04	2.31	2.51		0.29	0.31
	Passed by Centrifuge		1.06	0.57	1.67		0.54	1.58
		Sum	9.10	2.88	4.18	$\Sigma/\Sigma Ca$	0.32	0.46
	Retained by M.F.		11.60	2.26	5.56		0.19	0.48

This suggests that the centrifuge is effecting a separation of plankton species.

Other work, now in progress, shows that dinoflagellates are particularly rich in this compound and that they are thrown down by centrifuging less readily than diatoms and other plankton organisms. The samples in Table III contained an abundance of dinoflagellates, and the enrichment of chlorophyll *c* in the material passed by the centrifuge could reflect the failure of the instrument to retain dinoflagellates or fragments of them. Similar though less marked changes in the ratios of chlorophyll *b* to *a* could be interpreted as a selective failure of the centrifuge to retain small green algae.

LITERATURE CITED

1. Bush, J. H.
   1952. A basic new tool in bacteriology. Sci. Mon., *75* (5): 303–305.
2. Goetz, A. and N. Tsuneishi
   1951. Application of molecular filter membranes to the bacterial analysis of water. J. Amer. Wat. Wks. Ass., *43* (12): 943–984.
3. Goldberg, E. D., M. Baker, and D. L. Fox
   1952. Microfiltrations in oceanographic research. I. Marine sampling with the molecular filter. J. Mar. Res., *11* (2): 194–204.
4. Lovell Chemical Company
   (undated) Characteristics and uses of the MF Millipore filters. Lovell Chemical Company, Watertown, Massachusetts. Brochure, 22 pp.
5. Richards, F. A. with T. G. Thompson
   1952. The estimation and characterization of plankton populations by pigment analyses. II. A spectrophotometric method for the estimation of plankton pigments. J. Mar. Res., *11* (2): 156–172.

DIRECT MEASUREMENT OF THE HEMISPHERIC POLEWARD FLUX OF WATER VAPOR

By

VICTOR P. STARR

Massachusetts Institute of Technology, Cambridge, Mass.

AND

ROBERT M. WHITE

Air Force Cambridge Research Center, Cambridge, Mass.

ABSTRACT

The average meridional flux of water vapor in the atmosphere is evaluated for the northern hemisphere from suitably distributed daily observations of wind and moisture during the year 1950. Zonal averages for five latitude circles are presented, and these are compared with the corresponding requirements calculated from estimates of zonally averaged precipitation and evaporation given in climatological literature. Considering the nature and scope of the work, the extent of the agreement obtained is considered noteworthy.

INTRODUCTION

For several years the writers have been engaged in a long-term program of systematic compilation and analysis of hemispherically distributed observations of wind, temperature and moisture in order to elucidate the manner in which certain basic integral requirements of the general circulation are fulfilled in the atmosphere. As the studies proceeded, many of the results were published elsewhere (e.g., Starr and White 1951, 1952 a, b, c, 1954). However, the measurements relating to the poleward flux of water vapor have been completed only recently and have not been reported in their entirety save for some partial results contained in Starr and White (1954). The final outcome of the computations for the one complete year used (1950) is therefore presented herewith.

Since the divergence or convergence of the zonally averaged meridional flux of water vapor during a period of one year must reflect an excess or deficit of evaporation as compared to precipitation aside from minor secondary considerations, the present subject offers for the first time an independent means of checking the global precipitation and evaporation balance elaborated by various workers in the science of climatology during the past several decades. The impor-

tance of this entire field of endeavor for a number of oceanographic considerations hardly needs to be dwelt upon here.

PROCEDURE FOLLOWED

Water may be transported by atmospheric circulations in the solid, liquid and vapor form. For the purposes of our study, the transport in vapor form only is considered, the probability being that contributions from the solid and liquid forms are small in comparison, except as noted later. The flow of water vapor northward across a conical vertical wall along a complete circle of latitude from the surface to great heights and for any desired time period may be expressed as an integral of the form

$$\frac{1}{g}\int\int\int q\, v\, dx\, dt\, dp\,, \tag{1}$$

where g is the acceleration of gravity, q the specific humidity, v the northward component of velocity, dx an element of linear eastward distance, dt an element of time and dp an element of pressure taken vertically. The assumption of hydrostatic equilibrium is made use of in formulating this expression.

In recent years it has become feasible to evaluate the water vapor flux integral by using direct wind and moisture observations from a hemispheric network of upper air stations. While the quantity and quality of these observations are not yet adequate for reliable evaluation of the instantaneous transports on individual days, the mean flux over a large number of days can be approximated rather satisfactorily. This situation is comparable to the success with which the meridional flux of other quantities, notably of angular momentum, was measured by us in previous work to which reference has already been made. Certain details of the technique common to these studies, together with discussions of specific questions concerning the data used and other pertinent matters, are also to be found there. In view of this circumstance, only a brief statement of the methods is entered here.

The geographical distribution of the key stations is shown in Fig. 1 and is substantially the same as that used for the investigation of the hemispheric angular momentum balance (see Starr and White, 1954). This station network was again divided into five latitude zones centered in the vicinity of 13°, 31°, 42.5°, 55° and 70° N, as indicated.

The measurements of specific humidity were obtained from the reported dewpoints and temperatures. Because of the low moisture

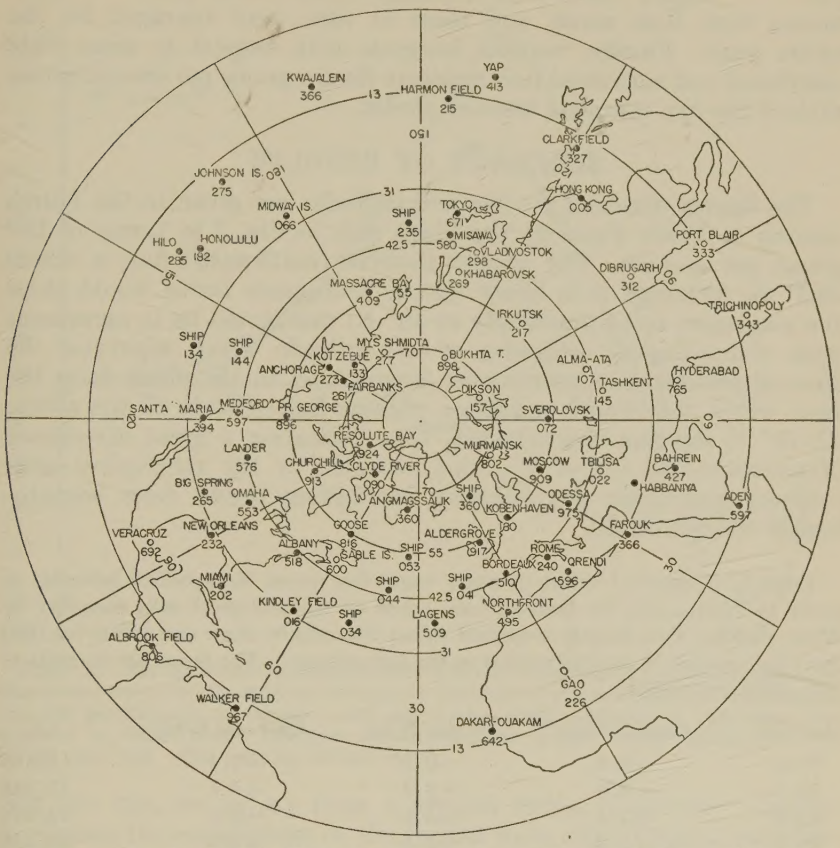

Figure 1. The distribution of key stations over the northern hemisphere used in the investigation of the atmospheric water vapor flux. Pilot-balloon wind stations shown by open circles, radio wind stations by dots.

content above 500 mb, the computations were restricted to the four standard pressure levels, 1000, 850, 700, 500 mb.[1] The flux of water vapor was evaluated at each latitude and level by first forming the product of the northward component of the wind and the specific humidity at each station for each day. Simple longitudinal daily

[1] In the processes of making radiosonde observations of humidity, an instrumental phenomenon known as "motorboating" occurs under certain definite atmospheric conditions. It indicates in general that the moisture content is too low to be measured and takes place at quite low temperatures ordinarily. An upper limit to the moisture amount may still be specified, however, and such values were used in these cases (see appropriate instructions for observers).

means were then made, and these in turn were averaged for the entire year. Finally, vertical integrals with respect to mass were calculated and converted into moisture flux in grams per second across each of the five complete latitude circles.

SUMMARY OF RESULTS

The figures obtained for the five latitudes are given in the fourth column of Table I and by the black dots in Fig. 2 in terms of 10^{11} grams per second. The largest positive (northward) flux is across 42.5° N, although it is likely that a continuous curve would show the maximum to be practically at 40° N; this would be in agreement with climatological estimates of the latitude which separates the zone of precipitation excess over evaporation to the north from the zone where the reverse condition obtains immediately to the south. At 13° N the flux is southward, thus indicating a great divergence of moisture flow out of the zone occupied by the subtropical anticyclones and again corroborating conclusions drawn from climatological considerations.

Table I. Numerical values of water vapor flux across the specified latitudes as given by Conrad and by Benton, both based on climatological data compiled by Wüst, together with directly measured values obtained by Starr and White for 1950 from the number N of observations in the last column. The fluxes are in units of 10^{11} grams per second.

Latitude	*Conrad-Wüst*	*Benton-Wüst*	*Starr and White*	*N*
70.0	+0.8	+0.5	+1.4	8,463
55.0	+3.6	+3.2	+4.4	12,845
42.5	+7.5	+5.5	+5.6	14,916
31.0	+6.2	+3.5	+4.6	20,748
13.0	−4.0	−4.6	−2.9	12,729

Numerical estimates of the water vapor flux requirements have been given by Conrad (1936) and by Benton and Estoque (1954), both of these being based on original data concerning precipitation and evaporation presented by Wüst (1922); these are shown in the first two columns of Table I and by the two curves in Fig. 2. Perhaps with some reservations relative to the situation in the tropics, which will be treated further below, it appears that agreement of measurements with requirements as portrayed by the curves is all that could reasonably be expected.

Since only five latitudes were sampled in making the computations, values of the flux at regular ten degree intervals cannot be interpolated without a certain subjectivity. Nevertheless, when this is done as

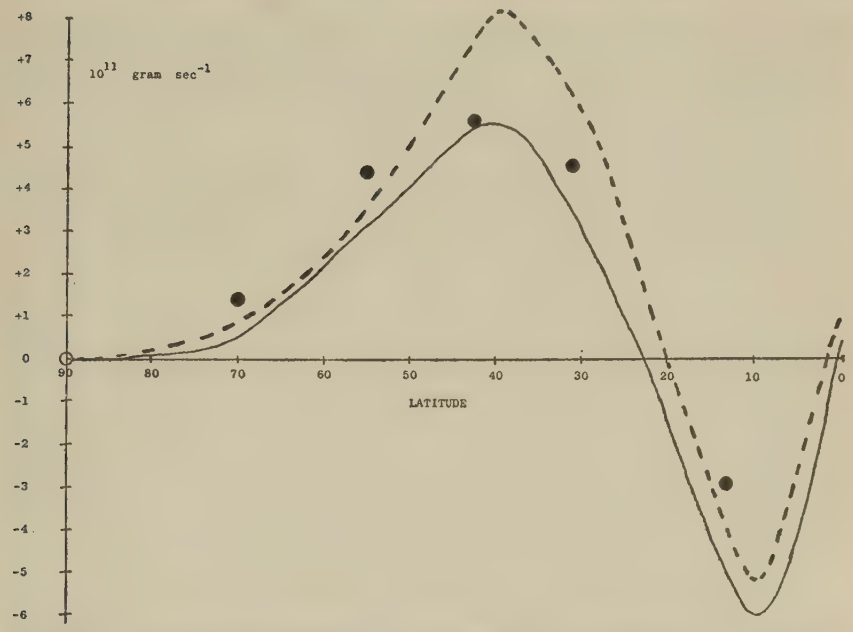

Figure 2. The meridional distribution of the poleward water vapor flux in the atmosphere. The dashed curve and the solid curve represent estimates of this flux deduced from evaporation and precipitation by Conrad (1936) and Benton (1953) respectively, both after data compiled by Wüst (1922). The dots represent the flux computed from atmospheric data for the year 1950. The units are 10^{11} gm sec^{-1}.

best one can, let us say from a smooth curve, it becomes possible to express the comparison of the results with climatological information in terms of the depth in centimeters of precipitation minus

Table II. The meridional distribution of the zonally averaged difference between precipitation minus evaporation by ten degree latitude belts for the northern hemisphere. The figures according to the indicated investigators are given in terms of cm per year.

Latitude Belt	*Conrad-Wüst*	*Benton-Wüst*	*Starr and White*
90–80°	+ 8	+16	—
80–70°	+22	+14	+27
70–60°	+25	+25	+35
60–50°	+32	+22	+19
50–40°	+32	+19	+ 7
40–30°	−19	−24	−11
30–20°	−48	−35	−30
20–10°	−37	−34	−34
10– 0°	+43	+46	—

evaporation on an annual basis by ten degree latitude belts. Such an arrangement of the comparison is given in Table II, where the figures obtained by the procedure here given are found in the fourth column while the requirements quoted from Conrad and Benton are found in the second and third columns.

It would add materially to the interest of Table II if the last entry were available. It is regrettable that upper air observation stations are not numerous enough as yet to permit water vapor flux measurements across the equator and thus provide this information.

SUPPLEMENTARY AND CRITICAL REMARKS

There are a number of points which may be raised concerning both the data and the computation techniques which doubtless contribute to the discrepancies between the three sets of results. It would scarcely be fitting or convenient in a discussion of this scope to engage in an evaluation of the techniques used by Wüst, Conrad and Benton. Therefore only certain of those factors which may lead to inaccuracies of the present calculation are touched upon here; specifically the more important considerations are the following:

(a) The direct computations of the flux are for the single specific year 1950, while the figures quoted from Wüst, Conrad and Benton represent long-term normals. It is highly probable that significant departures from normal do occur in individual years, although it is unlikely that the essential character of the meridional distribution of the water vapor flux changes radically from year to year. On the whole it would be highly coincidental if the flux distribution for 1950 were exactly normal.

(b) Fig. 1 shows the distribution of key stations used. In addition to these, numerous alternate stations were added as is described in the references already given. Since a certain fraction of the wind reports were obtained from pilot-balloon soundings, it is to be expected that some bias might thus be introduced because of the impossibility of making such soundings when cloudiness obscured the balloons. At least in middle latitudes this factor might lead to fluxes which are spuriously small, since the cloudiness is more prevalent on the eastward (more moist) sides of cyclones. Actually it is rather easy to overstress the importance of this circumstance, because the factors involved do not possess sufficient regularity. From an examination of the computations for various stations while the work was in progress, the impression is gained that not much error results from this source for the station network actually employed.

Another feature of the station network is that, generally speaking, it is more dense over land areas. This could lead to an insufficient

sampling of conditions over ocean areas where the moisture content is larger. It is not thought, however, that this factor has a very appreciable effect on the results.

(c) The assumption that the surface pressure has everywhere the constant value of 1000 mb is probably the most serious one made in the study. However, it is not unremediable, although its removal would involve a considerable increase in labor. The importance of this factor is probably greatest in the tropics, where surface winds having a component toward the equator are found in the mean and where the normal pressure is in the vicinity of 1010 mb, thus no doubt leading to an underestimate of the southward flux of water vapor. This same condition in the tropics probably represents also an instance where the effect of the transport of water southward in the form of liquid cloud droplets may be of some importance as well.

In terms of relative significance it appears that the neglect of the contributions to the flux from the atmosphere above 500 mb is of minor importance.

APPENDIX

It has been our custom in previous articles to present the results of flux calculations of various quantities in the form of a special table giving various details as to the classification of the atmospheric eddies which accomplish the transport. In the case of the flux of water vapor, such a table has been presented for each of the four latitudes 31°, 42.5°, 55° and 70° N by Starr and White (1954). In order to complete the series, the appropriate corresponding information for 13° N is here given in Table III. For a complete discussion of the terminology and symbols appearing in the column headings, see Starr and White (1954). For the purpose at hand it suffices to indicate that square brackets signify averaging with respect to longitude, a bar signifies averaging with respect to time, curly brackets indicate averaging over the total number of observations N, while n is the number of days with available observations and r is the coefficient of linear correlation between the northward component of wind velocity v and the specific humidity q. Primes denote the deviations from time or longitude averages, depending on the quantity to which they are affixed.

Note that, in the case of water vapor transport, the contribution of the so-called meridional cell component as given in column 6 at 1000 mb (due to net southward air motion) is large enough to give dominant importance to the vertical integral at the foot of the column. This is apparently due to the large concentrations of water vapor

Table III. Numerical analysis of water vapor flux data for year 1950 at latitude 13°N. All velocities are in m sec^{-1}, humidities in gm kg^{-1}. The levels are in mb.

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Level	$\overline{[q]}$	$\overline{[v]}$	$\overline{[q]\,[v]}$	$\overline{[qv]}$	$[\bar{q}]\,[\bar{v}]$	$\overline{[q]'\,[v]'}$	$\overline{[q'v']}$	*n*	$\{q\}$	$\{v\}$	$\{qv\}$	$\{qv\}-\{q\}\{v\}$	*r*	*N*
		*	*	*			*							
500	+ 1.6	−0.15	− 0.0	+ 0.4	− 0.2	+0.2	+0.5	365	+ 1.6	−0.16	+ 0.4	+0.7	+0.14	2873
		±0.18	± 0.3	± 0.3			±0.2							
700	+ 5.0	−0.06	− 0.1	+ 0.4	− 0.3	+0.2	+0.8	365	+ 5.1	−0.08	+ 0.6	+1.0	+0.10	3461
		±0.14	± 0.7	± 0.7			±0.3							
850	+ 9.5	−0.12	− 0.5	+ 0.7	− 1.1	+0.6	+1.2	365	+ 9.6	−0.11	+ 0.7	+1.8	+0.15	3171
		±0.14	± 1.3	± 1.4			±0.4							
1000	+15.6	−1.08	−15.7	−13.8	−16.9	+1.1	+2.0	365	+15.7	−1.05	−13.4	+3.1	+0.22	3224
		±0.19	± 2.8	± 2.9			±0.5							
Integral (10^2 CGS units)				− 0.7	− 1.6	+0.3	+0.6				− 0.7	+0.8	Sum	12729

* Confidence limits are twice the standard error of the mean.

near the surface and is in striking contrast to the situation in regard to the flux of angular momentum where the corresponding integral accounts for only a small percentage of the total flux which is itself small when compared to latitudes a little farther removed from the equator (see Starr and White, 1952c).

REFERENCES

Benton, G., and M. A. Estoque

1954 Water vapor transfer over the North American continent. J. Meteorol., *11:* 462–477.

Conrad, V.

1936. Die klimatologischen Elemente und ihre Abhangigkeit von terrestrishen Einflussen. Handbuch der Klimatolgie, Ed. Koppen and Geiger, Band I, Teil B, "Die Verdünstung." Berlin, Verlag von Gebrüder Borntraeger, pp. 360–362.

Starr, V. P., and R. M. White

1951. A hemispherical study of the atmospheric angular momentum balance. Quart. J. R. met. Soc., *77:* 215–225.

1952a. Note on the seasonal variation of the meridional flux of angular momentum. Quart. J. R. met. Soc., *78:* 62–69.

1952b. Schemes for the study of hemispheric exchange processes. Quart. J. R. met. Soc., *78:* 407–410.

1952c. Meridional flux of angular momentum in the tropics. Tellus, *4:* 118–125.

1954. Balance requirements of the general circulation of the atmosphere. Geophysical Research Papers, Air Force Cambridge Research Center (in press).

Wüst, G.

1922. Verdünstung und Niederschlag auf der Erde. Z. Erdkunde, *37:* 35–42.

ON THE AMINO ACIDS OF SPONGES[1,2]

By

LOIS WILEY INSKIP[3] AND HAROLD G. CASSIDY

Sterling Chemistry Laboratory, Yale University

ABSTRACT

The distribution of amino acids in 14 sponges was surveyed qualitatively by paper chromatography. Dried acetone-extracted sponge material was treated with 95% alcohol to remove soluble amino acids and peptides. From two sponges this material consisted largely of peptides, five of which were hydrolyzed and examined. From the present data it appears doubtful that any significant correlation exists between the amino acid distribution in sponges and the biological classification of these organisms.

Introduction. For some years in this laboratory, Bergmann (1949) has been conducting a comprehensive program including investigations of the lipoidal constituents of marine organisms. Among the material under investigation is an extensive collection of sponges which de Laubenfels (1936) identified and named and which Dr. Bergmann has placed at our disposal. We have examined the protein and other amino acid-containing materials in 14 of these sponges in a qualitative and semiquantitative manner with the following objectives in view: (*1*) to see if the amino acids present fall into any characteristic patterns; (*2*) to determine if any new or unusual amino acids or related substances may be present in the sponges (Ackermann and Burchardt, 1941; Ackermann, et al., 1924; Low, 1949); (*3*) to obtain data which might guide quantitative investigations.

An examination of this kind is made practicable through the use of the paper chromatographic method (Consden, et al., 1944; Gordon, et al., 1943). A preliminary survey of the applicability of the method to a number of sponges has been made by Hardy (1951).

Experimental. The sponge collection as received had been dried (usually in the sun) and the lipoidal material had been thoroughly

[1] Contribution No. 1155 from the Sterling Chemistry Laboratory, Yale University.

[2] Communication No. 34 in the series, Chemistry of Marine organisms. This investigation was supported in part by a grant from the Division of Research Grants and Fellowships of The National Institute of Health, U. S. Public Health Service, RG 1019.

[3] The contents of this paper are taken from the dissertation submitted by Lois Wiley Inskip to the Graduate School of Yale University in partial fulfilment of the requirements for the degree of Doctor of Philosophy, 1952.

extracted with acetone. The samples retained the skeletal material (keratinous, calcareous, or siliceous), the protein and other water-soluble components, as well as various inclusions such as sand grains, etc. These last were removed to the greatest extent possible and the sponge was then finely ground.

With the water-alcohol azeotrope, in a soxhlet, a known weight of air-dried sponge tissue was extracted continuously for several days until no further amino acid was being extracted (by the ninhydrin test). The extract was concentrated and made to 50 ml. Aliquots were taken for nitrogen determination. The remaining extract was then evaporated to a small volume and fractions were chromatographed. In two cases it was necessary to hydrolyze the extracts and chromatograph the hydrolysates because, along with free amino acids, the extracts contained peptides.

The residues from the alcohol extractions were dried in air and analyzed for ash, moisture (volatile at 125° in two hours) and nitrogen. Acid hydrolysis was carried out in 20% HCl for about 25 hours at 100° C. The excess acid was largely removed by evaporation and the hydrolysate, after being made up to a suitable concentration (usually about 16 mg N/ml), was chromatographed. After the acid hydrolysate had been applied to paper, the spot, while still wet, was exposed to concentrated ammonia to neutralize bound hydrochloric acid. Alkaline hydrolysis was achieved in a sealed evacuated tube at 100° for about 24 hours with room-temperature-saturated barium hydroxide. The barium was removed by passing carbon dioxide into the hot solution until no further precipitation occurred. After centrifuging off the barium carbonate and washing it with hot water, the hydrolysate and washings were brought to a concentration of about 16 mg N/ml and chromatographed.

Chromatography was carried out with strips or sheets of Whatman no. 1 paper, usually using with the sheets the phenol-"collidine" system, and upward development (Cassidy, 1951). The "map" published by Dent (1948) was used to assist recognition of the spots. Identification was also aided by calculation of relative R_F values and by use of known amino acids for comparative purposes.

In these sponges most of the common amino acids were recognized though not all were present in every sponge. By using numbers in the Tables, an attempt is made to indicate for a given sponge the relative amount of amino acid present as judged from the size of the spot and the intensity of the color in the given chromatogram. The numbers run from 0 for no apparent spot to 3 for large or intense spots. Comparison *between* sponges would not be legitimate from these tables, but paper chromatography is sufficiently developed

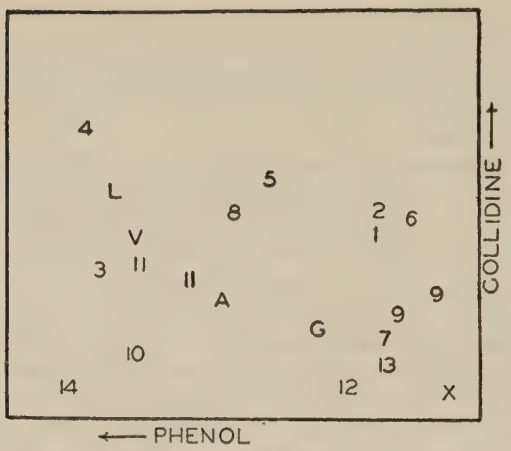

Figure 1. The relative positions of unidentified spots. G = Glycine; A = Alanine; V = Valine; L = Leucine. X point of application.

as a tool for identification so that the *known* amino acids could be identified with almost complete certainty. However, there were in the chromatograms a number of zones that could not be identified with certainty. The average relative locations of these, together with those of reference amino acids, are shown on a composite map in Fig. 1. Some of these unidentified materials appear to be present in quite large quantities in some of the hydrolysates.

Data are presented in Tables I to III. Table I adds to our knowledge of sponge composition and enables us to calculate the amount of "protein" in these materials. The nitrogen values were helpful in judging amounts of the extracts to be used in chromatography (Cassidy, 1951).

Every sponge extract contained glycine, alanine, valine, leucine(s), taurine and aspartic acid. One or more of the extracts also contained β-alanine, proline, methionine sulfoxide, glutamic acid, threonine, phenylalanine, tyrosine, lysine, arginine, ornithine, ethanolamine, hydroxyproline. Two sponges, *Tedania ignis* and *Aaptos* sp., contained peptides. These were examined as described below and the results are given in Table II.

For the purpose of determining whether or not the treatment of the sponge collection by sun-drying and acetone extraction produced extensive decomposition and loss of amino acid, a fresh specimen of *Microciona prolifera* was obtained through the courtesy of Professor Bergmann. This material was preserved by deep freeze and appeared to be in excellent condition. One portion was thawed and dried in

TABLE I. Gross Composition of Sponges, Ethanol-extractable Fractions, and Residues in Percent

		Solids Extracted by Ethanol		*Residue (air-dried)*		
*Sponge**	*N in air-dried tissue*$^{\Delta}$	*Air-dried tissue*$^{\Delta}$	*N in Solids*	*Moisture*	*Ash*	*Nitrogen*
1 Dysidea crawshayi†#	(6.54)			(5.2)	(41.2)	
2 Verongia fistularis†	6.48	14.5	4.7	7.1	19.8	7.3
3 Haliclona variabilis	6.72	26.4	8.8	7.9	17.3	8.8
4 Haliclona viridis	5.80	30.1	4.5	11.1	29	5.2
5 Lissodendoryx isodictyalis	5.61	32.7	3.8	9.7	32.6	6.1
Microciona prolifera						
6 Sun-dried	5.16	8.4	5.0	9.6	44.4	5.4
7 Freeze-dried	5.37	10.4	5.2	9.8	46.6	5.0
8 Tedania ignis	3.50	30	3.4	6.1	43	3.6
9 Axinella rosacea	4.08	38.8	2.3	9.2	33	4.3
10 Axinella rudis	4.84	29.4	3.1	12.5	34	4.7
11 Aaptos sp.	8.23	21.6	7.9	10.6	22.3	7.7
12 Suberites compacta	2.27	16.2	1.7	4.4	76	2.0
13 Terpios fugax	8.11	32.2	7.4	10.6	20.1	9.0
14 Cinachyra cavernosa	4.39	21.5	4.0	7.8	49	4.8
15 Leucetta floridana‡	0.88	14.0	1.6	0.9	89.0$^{\beta}$	0.8

* All are siliceous, except as noted.
\# Data from Hardy (1951).
† Keratinous sponge
‡ Calcareous sponge
$^{\beta}$ For determination of the calcareous ash, the sample was digested with 40% sodium hydroxide for 24 hours and filtered; the residue was thoroughly washed with water, dried and weighed. By the usual ignition process the ash yield was 45%.
$^{\Delta}$ Except sponge No. 7, which was freeze-dried.

the sun for eight hours at New Haven (probably a longer period of exposure than was needed at the collecting grounds); the second portion, kept frozen, was freeze-dried. Each portion was extracted separately in a soxhlet for 24 hours with acetone to remove lipoidal material. These extracts were then evaporated just to dryness and extracted with a small quantity of water; in chromatography they revealed traces of glycine, alanine, valine and leucine. Since these amino acids are present in the sponges in relatively large amounts, the loss incurred by acetone extraction in the collection procedure is considered to be insignificant.

The separate residues were treated in the same manner as the other sponge samples. The distribution of the known amino acids was identical in both sun-dried and freeze-dried fractions. Several zones which may have been present (but in some cases obscured) in the sun-dried fraction showed up only faintly in the freeze-dried fractions: spots *6* and *13* (Table I) in the acid hydrolysate; a methionine sulfoxide spot and the "unknowns" *4*, *6*, *8* and *12* in the alkaline hydrolysate. It was concluded that the method of collection introduced into the results no artifacts of any significance to this study.

TABLE II. Analyses of Peptides from Sponge Extracts

	Peptides				
	Aaptos sp.				*Tedania ignis*
Amino Acids	*A*	*B*	*C*	*D*	*E*
Glycine	1	1	1	?	3
Alanine		2	1	?	1
β-Alanine	1	1	2		
Valine		1	1	?	1
Leucine				?	
Serine	2				
Methionine sulfoxide	1			?	
Aspartic acid		1	1	1	1
Glutamic acid	2	1	1	1	2

A preliminary examination of the peptides in the alcoholic extract of *Aaptos* sp. was made. When the chromatogram from the unhydrolyzed extract was sprayed with 0.01% ninhydrin solution, four spots appeared which were not those of known amino acids and which disappeared from the hydrolyzed extract. These were designated *A*, *B*, *C*, and *D* (see Table II); only these appeared to contain enough material for further examination although there were other unknown spots and some known amino acids. A peptide *E* was similarly detected in the alcoholic extract of *Tedania ignis*. These fractions were prepared in sufficient amount by running a number of identical chromatograms, visualizing the spots with dilute ninhydrin, cutting out the spots and eluting according to the technique of Consden, et al. (1947). The data are given in Table II.

Table III shows the composition of the sponge residues (the proteinaceous tissues) left after alcohol extraction of free amino acids and peptides. The residues were subjected to both acid and alkaline hydrolysis, as described above. In alkaline hydrolysis the same pattern of composition as that in acid hydrolysis usually appeared except that there were some differences in relative intensities. For example, phenylalanine appeared in the alkaline hydrolysates of all sponges, but in the acid hydrolysates it was observed in only two of the sponges (Table III). Striking differences between the alkaline and acid hydrolysates were noted: the appearance of α-amino-*n*-butyric acid in *all* alkaline hydrolysates as well as intensified glycine and alanine spots with concomitant loss of intensity or complete disappearance of the serine and threonine spots. The alkaline destruction of serine and threonine has been noted frequently. Also observed was a faint monoiodotyrosine spot with the freeze-dried *Microciona prolifera;* a weak histidine spot with *Leucetta floridana;* and tryptophane in sponges *4* to *13* inclusive (see Table I). A number of the unidentified spots in the acid hydrolysates (Table III) were not found in the alkaline hydrolysates, and vice versa.

Discussion. Apart from the presence of the substances in the hydrolysates which produced the unidentified spots, the sponge proteins seem to contain a more or less normal amino acid distribution. That is, there is no overwhelming preponderance of one particular amino acid. The normal distribution also holds for the hydrolyzed extractives of the two sponges which contained extractable peptides. Histidine was found in only one of the sponges.

Taurine and ethanolamine were found in the ethanol extracts and in the two peptide-containing extracts as well. It is not considered likely that the taurine arose as a product of oxidation of cysteine, because no evidence of the extensive oxidation which would have been necessary to produce it appeared in a comparison of the freeze-dried with the sun-dried fresh sponge.

The presence of α-amino-*n*-butyric acid in alkaline hydrolysates of sponge tissue was observed by Hardy (1951). This amino acid appears in alkaline hydrolysates simultaneously with the disappearance of the threonine spot, and the disappearance of the serine spot in such hydrolysates was accompanied by an apparent increase in alanine and glycine. These observations agree with the well known degradation of hydroxy amino acids by barium hydroxide (Daft and Coghill, 1931).

The alcohol extracts of the two sponges *Aaptos* sp. and *Tedania ignis* yielded an atypical pattern of spots in chromatography. Since this pattern was reproducible, it suggested that peptides were present. Acid hydrolysis of an aliquot of the solution of the extract confirmed this conclusion when it yielded an hydrolysate which gave a normal pattern of spots on the two-dimensional chromatogram from which some of the original spots had disappeared. Isolation and hydrolysis of the material in some of these spots (see Table II) confirmed its peptide nature.

Since we did not isolate and identify by rigorous methods the substances responsible for the unidentified spots shown in Fig. 1, we do not suggest what they might be; we mention them only to suggest the possible course of further work.

In general, differences in amino acid distribution within each order of sponges are just as great as are differences between orders. From the present data it appears doubtful that any significant correlation exists between the amino acid distribution in sponges and the biological classification of these organisms. Most of the differences that were observed were in the unidentified spots, the common amino acids being almost always present. It is possible that elucidation of the chemical nature of the unidentified substances will reveal some interesting relationships, but it is unlikely that marked phylogenetic relations comparable to those reported by Bergmann and Feeney (1949) will be found within the amino acid distributions.

TABLE III. Analysis of Acid Hydrolyzed Sponge Residues. 0 = No Apparent Spot.
1, 2 & 3 refer to Spots of Increased Size and Intensity Relative to Each Other for a Given Sponge.

Amino Acids	*1 Dysidea crawshayi*	*2 Verongia fistularis*	*3 Haliclona variabilis*	*4 Haliclona viridis*	*5 Lissodendoryx isodictyalis*	*6 Microciona prolifera* Sun-Dried	*7 Microciona prolifera* Freeze-dried	*8 Tedania ignis*	*9 Axinella rosacea*‡†	*10 Axinella rudis*	*11 Aaptos* sp.*	*12 Suberites compacta*†	*13 Terpios fugax*	*14 Cinachyra cavernosa*	*15 Leucetta floridana*
Glycine	2	3	3	3	2	2	3	2	2	2	2	2	2	2	2
Alanine	2	3	3	3	2	2	2	2	2	2	2	2	2	2	2
Valine	2	3	2	3	2	2	2	2	2	2	2	2	2	2	2
Leucine(s)	2	3	2	3	2	2	2	2	2	2	2	2	2	2	2
Proline	2	2	2	2	2	1	2	2	2	2	2	2	2	2	1
Hydroxyproline	0	2	2	2	1	1	1	2	1	1	2	1	2	2	0
Serine	0	2	2	2	2	1	2	2	1	1	2	2	2	2	0
Threonine	2	2	0	2	2	1	2	2	1	1	2	1	2	2	1
Phenylalanine	0	2	0	2	2	2	1	1	2	1	2	1	2	2	2
Methionine	0	1	0	0	1	0	0	2	1	1	1	0	0	1	0
Methionine sulfoxide	0	1	0	1	2	0	0	1	1	1	0	0	1	1	0
Aspartic acid	2	2	3	2	2	2	2	2	2	1	2	2	2	2	2
Glutamic acid	2	2	3	2	2	2	2	2	1	1	2	2	2	2	0
Tyrosine	2	2	2	2	2	2	1	1	1	1	2	1	2	1	0
Histidine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lysine	0	1	0	2	1	1	2	0	1	1	1	1	1	1	1
Arginine	0	1	0	1	1	0	0	2	1	1	1	1	1	0	0
Ornithine	2	2	3	0	1(?)	2	2	1	0	0	2	2	0	0	1

Notes: The following amino acids were also present: *isoleucine 2; †isoleucine 1, citrulline 1; ‡ethanolamine 1.

The unidentified amino acids were present as follows: *1* in all sponges except 1, 3, 10, 14; *2* in 1, 2, 4, 8, 9, 10, 13; *3* in 4, 11, 12; *4* in 4, 5, 9 through 14; *5* in 10; *6* in 3, 7, 14; *7* in 5, 7, 9, 10, 12; *8* none; *9* in 3, 8; *10*, *11*, and *12* in none; *13* in 1, 3, 7, 10. It was not always possible to distinguish between unidentified spots *1* and *2*.

REFERENCES

Ackermann, D. and C. Burchardt

1941. Zur Kenntnis der Spongine. Hoppe-Seyl. Z., *271:* 183–189.

Ackermann, D., F. Holtz, and F. Kutscher

1924. Über die Extraktstoffe von Eledone moschata. Z. Biol., *80:* 155–162.

Bergmann, Werner

1949. Comparative biochemical studies on the lipids of marine invertebrates, with special reference to the sterols. J. Mar. Res., *8:* 137–176.

Bergmann, Werner and R. J. Feeney

1949. Contributions to the study of marine products. XXIII. Sterols from sponges of the family *Haliclonidae.* J. org. Chem., *14:* 1078–1084.

Cassidy, H. G.

1951. Adsorption and chromatography, Vol. V, in Technique of organic chemistry. A. Weissberger, ed. Interscience Publishers, New York. 360 pp.

Consden, R., A. H. Gordon and A. J. P. Martin

1944. Qualitative analysis of proteins: a partition chromatographic method using paper. Biochem. J., *38:* 224–232.

1947. The identification of lower peptides in complex mixtures. Biochem, J., *41:* 590–596.

de Laubenfels, M. W.

1936. A discussion of the sponge fauna of the Dry Tortugas. Publ. Carneg. Instn., 467. 225 pp.

Daft, F. S., and R. D. Coghill

1931. The alkaline decomposition of serine. J. biol. Chem., *90:* 341–350.

Dent, C. E.

1948. A study of the behaviour of some sixty amino-acids and other ninhydrin-reacting substances on phenol-'collidine' filter-paper chromatograms, with notes as to the occurrence of some of them in biological fluids. Biochem. J., *43:* 169–180.

Gordon, A. H., A. J. P. Martin, and R. L. M. Synge

1943. Partition chromatography of free amino-acids and peptides. Biochem. J., *37:* Proc. xiii–xiv.

Hardy, R. A., Jr.

1951. The amino acid composition of sponges. A study of paper chromatography. Dissertation. Yale University.

Low, E. M.

1949. Iodine and bromine in sponges. J. Mar. Res., *8:* 97–105.

SEA TEMPERATURE VARIATIONS ASSOCIATED WITH TIDAL CURRENTS IN STRATIFIED SHALLOW WATER OVER AN IRREGULAR BOTTOM[1]

By

Dale F. Leipper

Department of Oceanography, Agricultural and Mechanical College of Texas

ABSTRACT

Unusual features of the large and nearly periodic variations in sea temperature which are observed in shallow stratified water along the coast of southern California may be caused by tidal stirring over an irregular bottom and by subsequent horizontal and vertical oscillating movements associated with tides.

PART I

Sea temperature variations having an average diurnal range of approximately 4° C during summer months are indicated on thermograph records obtained at the end of Scripps pier in the years 1926 through 1931. Other observations show that cold spots are found in certain localities along the coast, that the vertical temperature structure changes markedly with position, that the amplitude of local temperature variations changes systematically from week to week, and that large local temperature changes occur in a short time. The records have been analyzed here to determine the time, the frequency and the range of the variations at different depths and in different months. Analysis of the data and further investigations led to a theory which, when considered together with effects of the normal wave action associated with tides (see Arthur, 1954), accounts for most of the features of the variations. This theory makes use of a formula developed by R. H. Fleming for tidal current velocities in shallow water. The formula indicates that, in certain regions, widely different velocities may be found adjacent to each other. The differences lead to formation of eddies which, over irregular bottom, cause stirring. In highly stratified water, such as that found off southern California in summer, this bottom stirring causes cooling in the upper layers or tends to intensify vertical temperature

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gradients in any overlying thermocline. Oscillating tidal currents then cause the "cold spot" thus formed to migrate back and forth so that large temperature variations are observed in any locality where the cold water mass, during its horizontal migration, replaces normal unstirred waters. The intensification of the temperature gradient in the thermocline also results in more marked local temperature changes associated with vertical oscillations of the water.

In the following discussion, the proposed theory and a description of the kind of predictions which it permits establish a framework into which the various supporting empirical features may be fitted. Further details may be found in a dissertation by Leipper (1950).

Since tidal currents vary according to the stages of the tide, it is convenient to fix attention upon velocities at a certain tidal stage. In the following, attention shall be centered on the tidal stage having the maximum tidal-current velocity.

Intuitively, one would expect the maximum velocity of tidal current near shore on a continental shelf to be proportional to the distance from shore and to be inversely proportional to the wave period and depth, i.e.

$$V_{\max} = \frac{kx}{Th},$$

where x is the distance from shore, h the depth, T the wave period, and k a constant. Such a relation may be developed from physical considerations.

By using an expression derived by Lamb (1932: 254–256) from the equations of motion and from the equation of continuity and by introducing certain assumptions which seem reasonable for the continental shelf, Fleming (1938) developed a method for computing the maximum tidal-current velocity. It is assumed that the tidal amplitude is nearly constant over the small area near shore, that the wave length is great compared to the distances under consideration, and that the wave is a standing wave with node line parallel to the coast. The expression for the maximum velocity of the tidal current is then given by equation (2), where the constant k in equation (1) becomes the product of 2π times the wave amplitude η

$$V_{\max} = \frac{2\pi\eta}{T}\frac{x}{h}. \tag{2}$$

A quantity C may be defined as

$$C = V\left(\frac{T}{2\pi\eta}\right). \tag{3}$$

That is, from equation (2), $C = x/h$.

When we examine charts showing details of bottom configurations in a band approximately five miles wide along the California coastline, we observe that many localities have configurations which, in the light of equation (2), lead to widely different tidal current velocities in areas not far removed from each other. Any locality where the spacing of the inshore bottom contours change suddenly along the coastline has associated velocity variations. Such changes in contour spacing are often observed where a point of land juts seaward. Different velocities in adjacent areas tend to cause a turning of the currents, the stronger one running ahead and turning toward the weaker; thus eddies are formed.

Mechanical stirring occurs in the presence of tidal currents. For example, Sverdrup, et al. (1946: 566–569), in discussing the effects of the bottom, have explained that currents must be deflected vertically or horizontally by submarine peaks. The stability of the water, the strength of the current, and the configuration of the peaks determine the amount and direction of deflection. Quantitative estimates of such modifications of tidal currents in shallow water and the effect of these modifications on the mixing processes have not yet been made.

Since the above difference between the velocities of maximum tidal currents is due to a difference in bottom slopes and since the resulting eddies form between the different currents, the eddies are formed over a region of changing bottom slope where they must have some associated vertical movement. Such movement intensifies both vertical and horizontal stirring of waters of different temperatures.

If the waters are nearly homogeneous, as is often the case in the upper layers during winter, stirring related to tidal currents has little or no effect in bringing about local changes in temperature. However, in summer the surface layers may become highly stratified. Stratification may be particularly marked in regions where cold upwelled water approaches the surface but where normal heating occurs at the surface. Under these circumstances any vertical stirring may markedly cool the surface layers. Thus, in summer we may expect a cold spot to develop over an area where bottom contours are favorable for variation in tidal currents, with consequent stirring and mixing. It is possible that the stirring action will occur in the underlying water only and will not reach the surface. In this case a shallow and more intense thermocline develops which may define a cold spot at some level below the sea surface.

In most localities the wave period of tides does not change significantly from day to day whereas the amplitude does. Since the maximum velocity of a tidal current is directly proportional to the

amplitude [eq. (2)], the local velocities also change from day to day. This causes changes in the intensity of eddying movements and in the amount of related stirring action. Thus, in any region where bottom contours favor the formation of eddies and where temperature stratification exists, it is to be expected that a cold spot will form and will increase in extent from day to day while the tidal range and the corresponding tidal mixing decreases.

As noted, the maximum tidal-current velocities at a given distance from shore are greatest where the depth is least. Thus the amount of stirring associated with these currents would be greater in shallow water. Also, in depths below the thermocline, the vertical temperature gradient is relatively small and a given amount of stirring here leads to smaller local temperature changes than at shallower depths where the temperature gradient may be larger. It is true that the greater stability in the thermocline region opposes vertical mixing. However, this is not significant in the face of the energy available in the currents related to the tides, and it may be expected that the largest temperature changes caused by tidal currents at a given distance from shore will occur in the shallowest waters where the vertical temperature gradient is largest.

Regions having large temperature changes associated with tidal stirring are limited in size. Limiting factors are: the dimensions of the area having bottom contours favorable for stirring; the size of the region having highly stratified water; and the distance which water migrates in a tidal cycle. Consideration of these factors leads to the conclusion that cold spots would be expected to have diameters not greater than approximately three miles.

Because of the nature of the currents in the region in which cold spots are formed, the spots must migrate or move with the tides. The astronomic tides are periodic, being semidiurnal in most regions, and the vertical and horizontal movements which are required to permit the rise and fall of the water level in tidal action are the tidal currents which affect the cold spots. These currents must also be periodic and oscillating, the periods being the same as those of the tides unless the currents are modified by other influences.

In a given locality the tidal current at a certain stage in one tidal cycle may be different from that at the same stage of other cycles. Differences occur which are related to the change in tidal amplitude and to the influence of varying local ocean currents not related to the tide.

Since cold spots are produced by stirring in tidal currents, they always exist in regions where they cannot remain stationary. They must move with these currents, migrating back and forth in a time

equal to a tidal period. The distance of such migration depends on the average tidal-current velocity. This is usually less than one-half mile per hour, thus limiting the migration of a cold spot in a semidiurnal tidal cycle to less than approximately three miles.

Seasonal or wind-driven currents which change their direction or speed but which are not related to the tides sometimes aid and at other times hinder the tidal migration of a particular cold spot toward an observation point. This accounts for the cold dip at a given locality occurring sometimes at one tidal stage and sometimes at another.

Since cold spots are formed by turbulence over an irregular bottom, their three-dimensional structure will be dome-like, with the broadest extent of mixed water and the lowest temperature in the deepest levels. As such a dome or crest moves toward a point of observation, decreasing temperatures will be noticed first at the bottom. As the dome pushes forward, the cold water will be observed at progressively shallower depths until the dome crest arrives at the observation point, at which time some of the original warm water mass may or may not remain above the dome. As the dome recedes, the warm water will return first at the surface and then will gradually occupy the greater depths.

The foregoing considerations permit the extrapolation of certain observed local temperature trends with greater accuracy than has been possible previously. For example, we may predict that the local temperature range in regions having cold spots will increase as stratification increases and that it will decrease as the waters of the surface layers become more homogeneous. Also, if temperature dips have been observed from day to day at a given tidal stage, we forecast that the next dip will occur at that same stage of the predicted tide, assuming that no significant change occurs in the wind or in other nonperiodic phenomena. In extrapolations, use may be made of day to day trends in the amplitude of temperature variations which are associated with increasing and decreasing tidal ranges.

Horizontal distributions of temperature in shallow water often may be explained or predicted by considering the tides, the water stratification, the bottom topography, and the tidal stirring process.

PART II

Data collected from 1916 to 1950 under the supervision of Dr. George F. McEwen have been used to study the validity of the model for temperature variation described above. These data include: daily sea-surface temperatures collected for more than 30 years at selected points along the coast; four years of thermograph records

taken 2 feet and 16 feet above the bottom at the end of the 1100-foot Scripps pier; hydrographic observations taken 5 miles and 11 miles west of the pier at two-week intervals over a period of several years. Following an analysis of these data, which brought to light several unusual and marked types of variation in the shallow-water temperatures, additional observations were planned and carried out over a two-year period to supplement the McEwen data and to provide information that was needed to serve as a further check on the explanation for the observed variations. (Bathythermograph [BT] records are in degrees Fahrenheit, the other records in degrees Centigrade; for convenient comparison with other data summaries, both of these respective units have been retained in this analysis.)

The quantity C, defined in eq. 3, has been computed for various locations off La Jolla, California by using a chart that shows bottom topography. Lines of equal C value (i.e., lines of constant relative theoretical velocity of maximum tidal current) have been drawn in Fig. 1. It is evident that there is a great variation in the value of maximum velocity with position. West of La Jolla, velocities are as much as 3½ times greater than those north and northwest of La Jolla. A similar chart prepared in greater detail showed velocities in one area which were 9 times greater than those in a nearby area.

To obtain observed values for comparison with theoretical current velocities, simultaneous drifts of two submerged triplane buoys in a 3-hour interval between low and high tide were carefully plotted. The buoys were released in regions that were computed to have quite different tidal-current velocities, the ratio between velocities being approximately 7 : 3. The two paths of drift are shown in Fig. 1. The ratio of two *total* drifts was 3.26 : 1, that of *net* drifts (distances from beginning to end points) 2.19 : 1. The theoretically computed ratio of 2.43 : 1 compares favorably with observation, and the theory is supported. The observed velocities were from 0.2 to 0.6 knots.

Drift observations were carried out by John Blankenship who took particular care to eliminate the effect of wind on the buoys. The day selected was November 10, 1948, with a low tide of 2.5 feet at 1248 PST and a high tide of 4.3 feet at 1746. Buoys, dropped one hour after low tide, were picked up 58 minutes before high tide. Note in Fig. 1 that the drift was generally toward the southeast while the tide was rising.

The significant features of the seasonal temperature variation are indicated by the temperature-depth structures in Fig. 2, in which curves for winter and summer are compared. In Fig. 2a the curves were obtained by averaging all available BT observations taken in

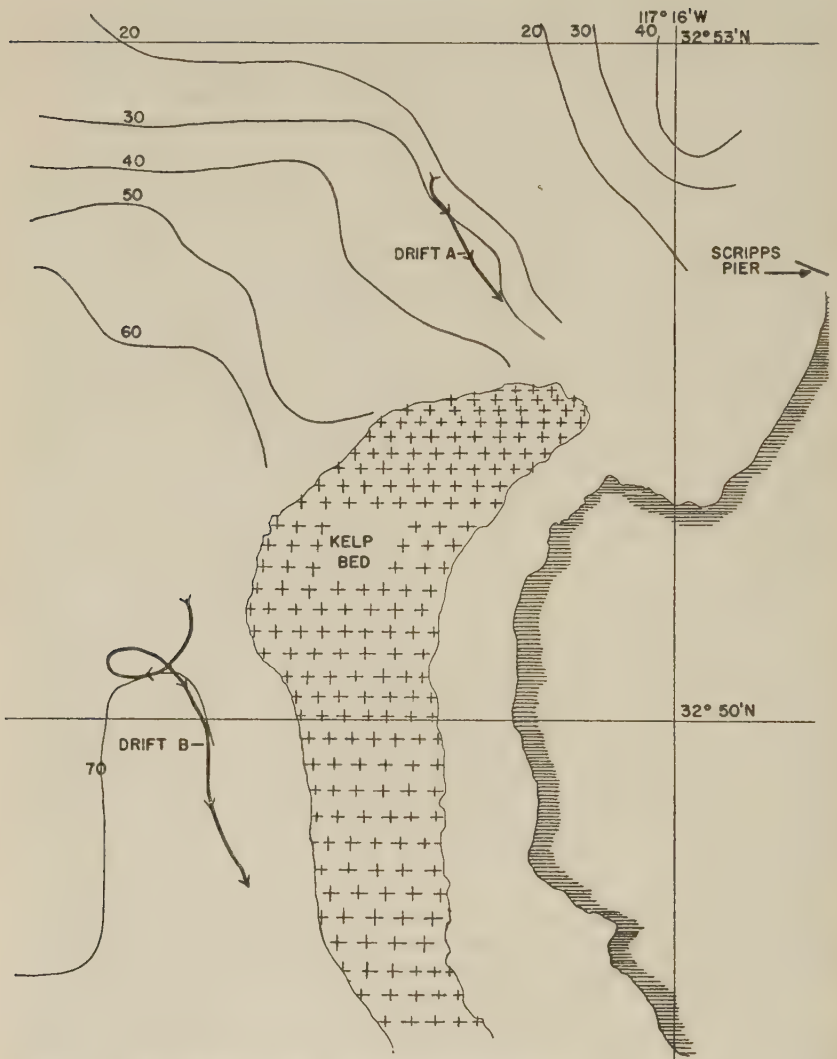


Figure 1. Simultaneous drifts of two submerged triplane buoys in a 3-hour interval between low and high tide; also, lines of constant relative theoretical velocity of maximum tidal current.

August and also in February from a region about 24 miles offshore. Above 100 feet the change from winter to summer is more or less typical of most ocean areas, with nearly isothermal water of low temperature in winter and more stratified water with higher surface temperature in summer. From 100 to 400 feet, however, the change is opposite that which might be expected in most regions, since the temperature in summer is lower than that in winter. This change is even more apparent in the example in Fig. 2b which is based on two single hydrographic observations made at the same place in March and

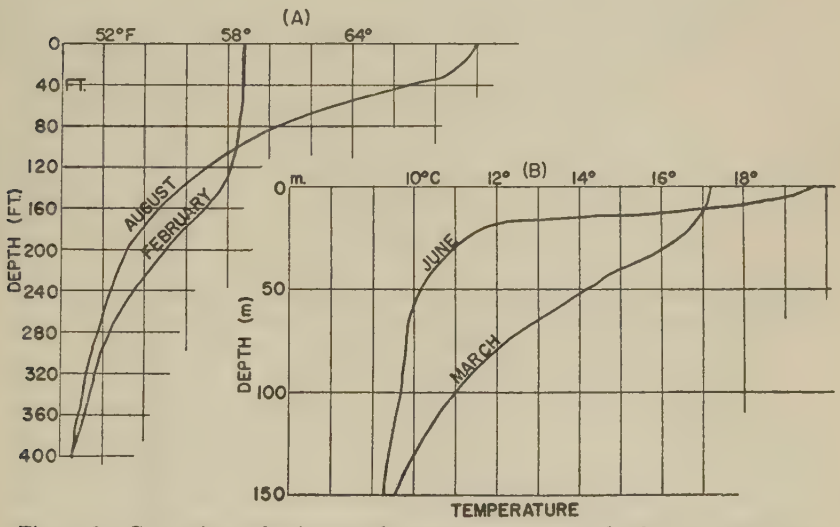


Figure 2. Comparison of winter and summer temperature-depth structures offshore near Scripps Pier.

June; all of the water below about 10 m dropped in temperature during the March–June interval. At about 30 m the temperature was about 5° C lower in summer than in winter. This type of variation, common to the region off southern California, has often been described (McEwen, 1916: 352–353, fig. 985). It has been explained that this phenomenon is due to upwelling brought about by strong northwesterly winds in the springtime north of Point Conception. After upwelling, this cold water flows southward and is gradually warmed through a thin layer near the surface; when it arrives in the southern California area, the temperature-depth structure in summer has the appearance of the June and August curves shown in Fig. 2.

The combination of normal surface heating and abnormal cooling

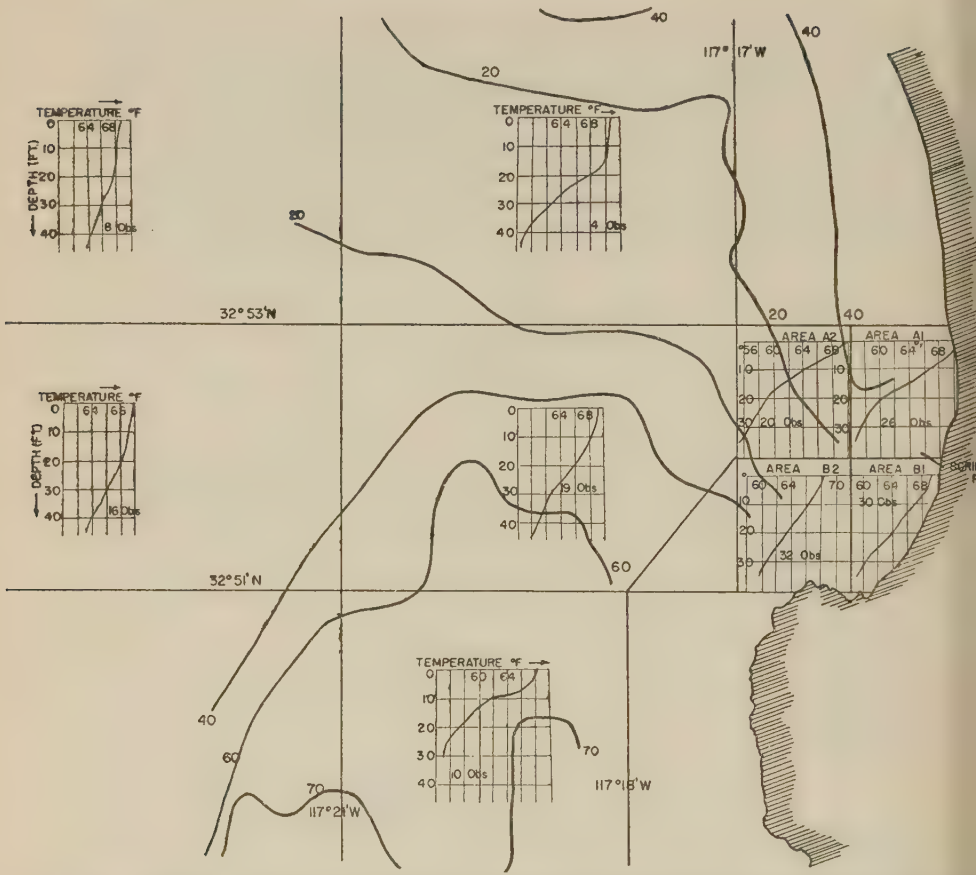


Figure 3. Average temperature-depth structure in summer in different areas near Scripps Pier; also, lines constant relative theoretical velocity of maximum tidal current.

which is indicated at some depths below the surface results in a sharp temperature contrast between the surface layers and those immediately below them. This contrast sets the stage for the development of local cold regions near the surface when the two layers are stirred in places by varying tidal currents.

Another important feature of the temperature distribution is that the temperature structure of the water near shore differs from that farther out. For example, Fig. 3 shows that the vertical temperature gradient in summer near the surface off Scripps pier is much greater than that farther offshore. For the 0 to 30-foot depth interval, the average temperature decrease with depth along the coast is always 8° F (at

times greater than 14°) for the regions shown, whereas in offshore areas the average decrease is only 3° F. Thus, along the coast a given stirring of the surface waters might result in a large temperature decrease, whereas farther offshore, where the water is more nearly isothermal, the same amount of stirring would change the surface temperature but little.

In winter, vertical temperature differences are small over the entire area, being usually less than 1° F per 30 feet of depth. Hence a given amount of stirring could result in only a small decrease in surface temperature; furthermore, the possible temperature drop brought about along the coast would not be appreciably different from that in the region offshore.

TABLE I. Average Monthly Diurnal Range of Sea Temperature at Scripps Pier (°C)

Month	*J*	*F*	*M*	*A*	*M*	*J*	*J*	*A*	*S*	*O*	*N*	*D*
2 feet above bottom	0.5	0.5	1.0	1.3	1.4	1.3	1.8	1.7	1.6	1.0	0.5	0.4
16 feet above bottom	0.6	0.7	0.9	1.2	1.2	0.9	1.6	1.3	1.3	0.9	0.6	0.6

On 19 days throughout the year, series of observations were made at one mile intervals on a line perpendicular to the coast extending 10 to 15 miles offshore. Temperature-depth sections of these data showed 18 sections with isotherms rising toward shore. This appears to be a general tendency.

To compute the average amplitude of the diurnal temperature variation on ordinary calendar days, values were read from the Scripps Pier thermographs at two-hour intervals beginning at midnight; monthly averages were then calculated for each hour read. The difference between the lowest and highest average for a given month is the diurnal range for that month. Monthly ranges were computed for three separate years, and these were then averaged together by months (see Table I).

Note two unusual features of the data in Table I. First, from March through October the diurnal range of variations near bottom is greater than the range 16 feet above bottom. Since solar radiation is absorbed rapidly near the surface and since only about 6% of it reaches a depth of 5 m or 16.8 feet (Sverdrup, et al., 1946: 107), an important diurnal process other than radiation is indicated. Second, the diurnal ranges in July near the bottom are large, reaching a value of 1.8° C or 3.2° F. The maximum average diurnal variation caused by solar heating is smaller and may be calculated approximately by assuming that the incoming energy is uniformly distributed by intense mixing throughout a surface layer 5 m in depth. Using the maximum average

insolation at the Pier, 484 g cal/cm^2 (McEwen, 1938: 236), the maximum daily increase in sea temperature at a depth of 5 m is 1° C. The increase which would be expected is considerably less than this because of heat lost by evaporation, conduction and radiation from the sea surface. Thus the observed variation is much larger than that due to the processes usually considered.

At the Scripps pier the temperature variation within a given day, strangely enough, is more regular in winter than summer. In sum-

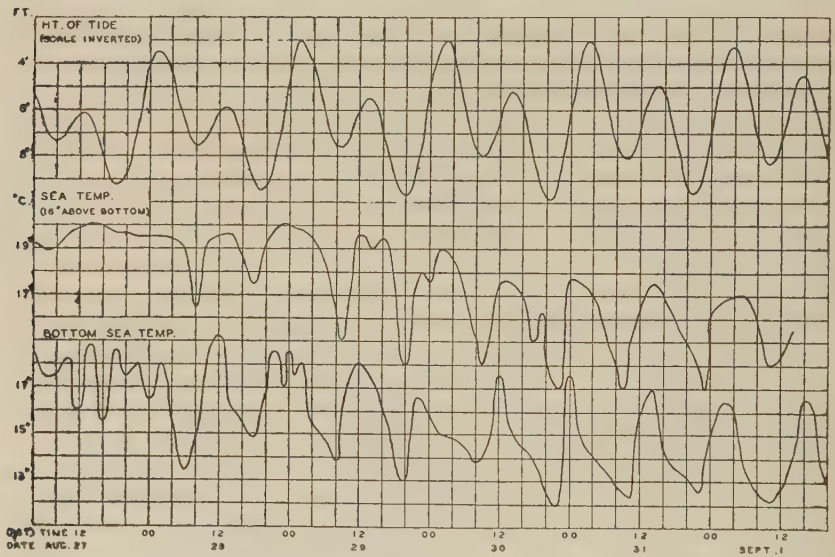


Figure 4. Tide and sea temperature variations at Scripps Pier, 1928.

mer it is masked by other types of temperature fluctuations, including a series of dips which usually occur daily at intervals approximately equal to tidal periods. See Fig. 4 for an example of temperature variations observed during a week in the summer of 1928 together with the associated heights of the tide. The dips shown on the thermograph traces do not have the appearance of sinusoidal variations; many of them are of the type which might be expected when one water mass is rapidly replaced by another of different temperature. In some cases, changes as great as 7° C occur in periods as short as 15 minutes. As these dips increase in size from day to day, the average temperature as well as the daily maximum at Scripps pier decreases (see Fig. 4).

Variations of the type shown in Fig. 4 are absent during winter, and their nature varies considerably from day to day and from week

to week during the summer months. In 1928 the lowest temperatures occurred regularly with the high tide. In other weeks, however, they occurred with the low tide. As will be shown, the former occurrence is more common, but cases where the coldest water is associated with every stage of the tide have been observed. Correlation by harmonic analysis should not be attempted because of the obvious shift in phase.

Tabulations show that the majority of maximum daily temperature dips occur during the night. Since these dips appear to be related to tidal currents, diurnal characteristics might be expected when it is considered that the solar tidal component is 43% of the lunar component and that tidal currents are subject therefore to a large diurnal influence. The regular occurrence of the larger nocturnal dips lowers the night-time average temperatures and thus increases the amplitude of the ordinary diurnal variation.

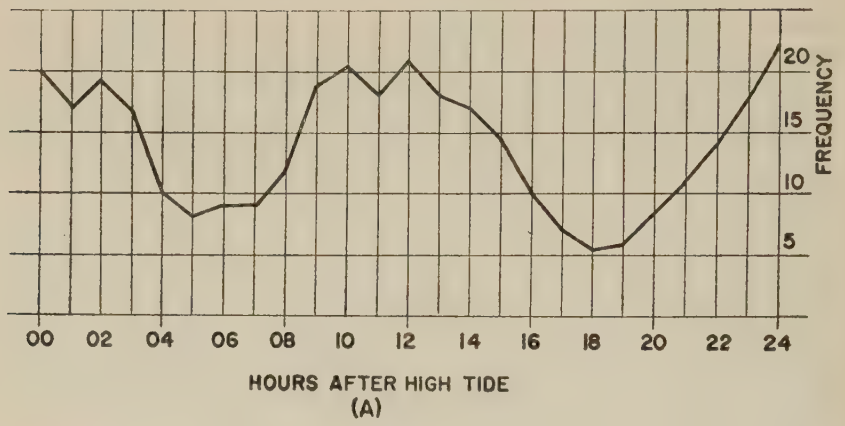
To study temperature variation within diurnal tidal periods, time was counted from one high tide to the second following high tide. In this manner 'tidal days' were defined as 24 to 25 hours in duration.

A frequency diagram in Fig. 5 shows the number of times that dips occurred on each hour of the tidal day. Fig. 5a is based on only the maximum dip which occurred each day, while Fig. 5b is based on all distinct dips, usually two for each day. Greatest frequencies were observed at approximately 0000 and 1200 h, which corrrespond to high tides. Least frequencies were observed at about 0600 and 1800, the times of low tides. Of the total number of dips, 55% occurred within two hours of the high tide while only 24% occurred within two hours of the low tide. For given series of successive days, the time of the dips did not shift erratically in the tidal time scale.

An effort has been made to determine under what conditions temperature dips may be expected at high tide and under what conditions they may be observed at other times. There appears to be a systematic seasonal deviation of the time of occurrence from the time of high tide as the year progresses (see Leipper, 1950).

To study further the tidal effects on temperature, the ordinary diurnal heating was eliminated. Average monthly temperatures were obtained for each 2-hour period of the day, and the sine curve of best fit was drawn through the 12 averages. It was assumed that this curve represented the ordinary diurnal variation; deviations from the monthly mean shown by this curve at various hours of the solar day were used as corrections to the observed temperature values to eliminate the diurnal effect.

On each tidal day the temperature range was obtained by subtracting the minimum from the maximum, using the adjusted data.



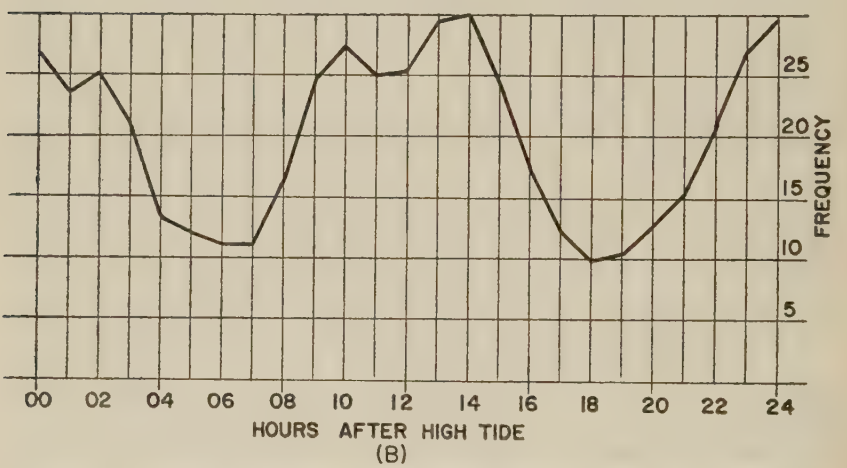


Figure 5. Frequency of occurrence of temperature dips at Scripps Pier in different one-hour intervals after high tide.

Ranges were then averaged by the month (see Fig. 6a). Data for the near-bottom changes are presented, since changes at this level are more marked and more consistent than those at the upper level. In winter the temperature range on tidal days is small, but the range increases in spring, and in summer it averages more than 4° C (see Fig. 6a). On the original thermograph traces it is difficult to observe any dips in December, January and February. During summer, dips have occurred on individual days which have lowered the temperature at Scripps pier below the lowest values observed in winter months.

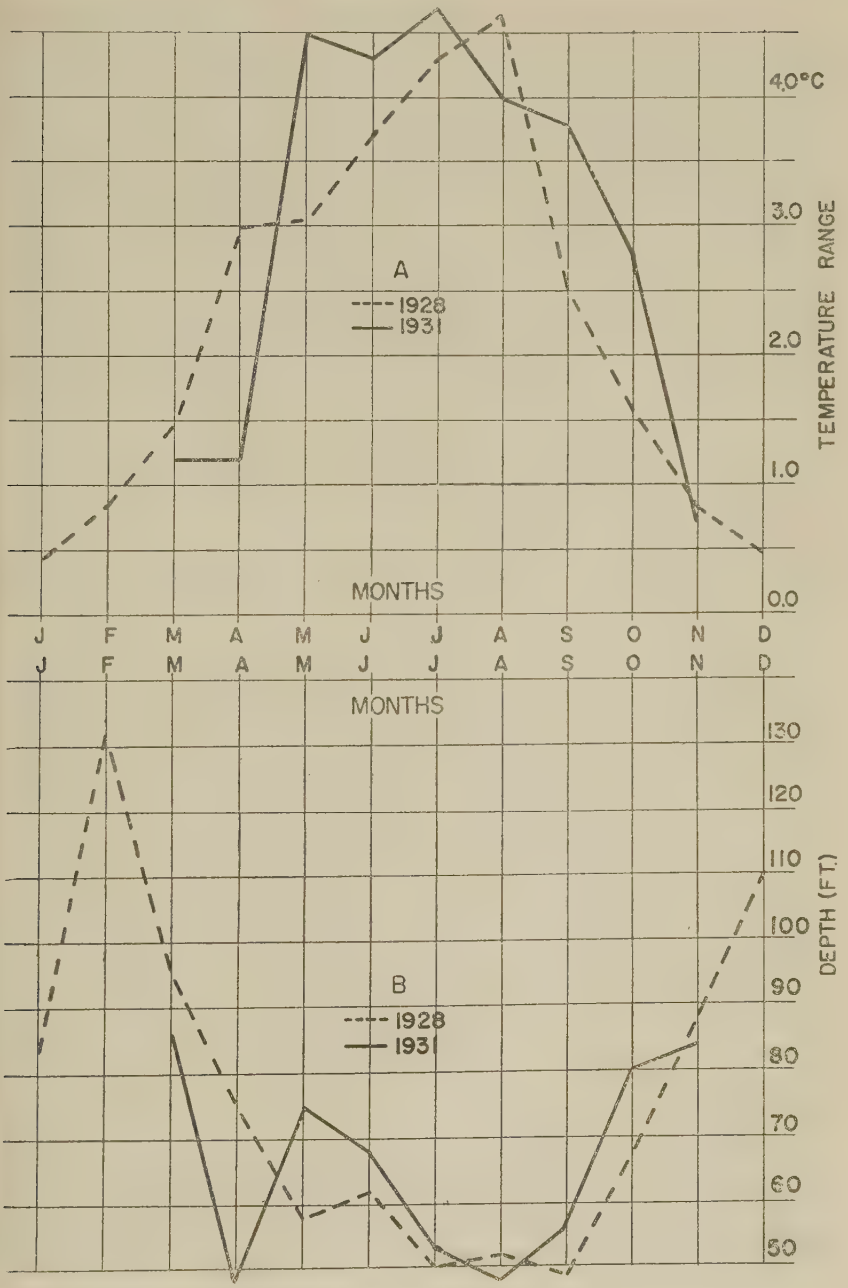


Figure 6. Monthly average range of sea temperature at bottom, Scripps Pier, on tidal days. Depth of water offshore which is colder by the amount of the average daily tidal deviation than the average observed at the bottom.

As noted, the presence of cold upwelled water near the surface in summer together with vertical and horizontal tidal movements can result in large local temperature dips. As shown in Fig. 6b, water as cold as that observed in the average cold dip in summer at the pier may be found within 50 feet of the surface off the San Diego area. (Offshore temperatures for this comparison were obtained by averaging BT observations taken about 24 miles from the coast.) Fig. 6b also shows that, although the dips in spring and fall lower the temperature an average of only about 1° C, water comparable to that which is colder than the average by one degree at the pier bottom cannot be found offshore in these seasons at less than 80- to 90-foot depths. Water with a temperature that is low enough to cause the extremely cold summer dips is closer at hand than that required to cause the moderate dips in fall and spring.

TABLE II. Ratios of Monthly Average Amplitudes and of Durations of Temperature Dips 2 Feet Above Bottom to Those 16 Feet Above Bottom

		J	*F*	*M*	*A*	*M*	*J*	*J*	*A*	*S*	*O*	*N*	*D*
Amplitude	1928	—	0.7	0.8	1.3	1.4	1.6	1.4	1.5	1.3	1.0	0.9	—
Ratio	1931	—	—	0.5	0.7	1.4	1.8	2.6	3.3	2.0	2.4	1.8	—
Duration	1928	—	0.8	0.9	1.1	1.2	1.4	1.3	1.3	1.0	0.8	0.8	—
Ratio	1931	—	—	0.3	0.8	1.1	1.4	1.5	1.8	1.5	1.6	1.0	—

The relative magnitudes of tidal changes in near-surface and near-bottom temperatures were analyzed by defining two ratios: (1) that of bottom to surface temperature change, called the 'amplitude ratio,' and (2) that of the duration of bottom dip to surface dip, called the 'duration ratio.' Ratios were computed from data which were not adjusted for diurnal variation, since the diurnal effects near surface and bottom were found to be nearly equal.

For 1928 and 1931, the amplitude ratio had minimum values in winter, maximum values in summer (see Table II). In winter the amplitude is greater near the surface than near the bottom, but in summer the situation is reversed and the near-bottom change may be more than three times that near the surface. The larger values of the 1931 ratio are probably associated with the fact that this was one of the warmest years on record; it is quite likely, therefore, that this was one of the years when the vertical temperature gradient was unusually high.

The trends of the duration ratio are similar to those of the amplitude ratio, but the variation is not quite so great. The monthly values of this ratio are also shown in Table II. Except when the deeper observation is at the depth of a strong thermocline and when internal waves are present, the amplitude and duration ratios for

most parts of the ocean would be less than unity, since nearly all factors that affect sea temperature have their maximum influence at the surface. The fact that the pier ratios are greater than unity demonstrates that interval waves, tidal oscillation, or both are present.

If the theory presented is applicable at Scripps pier, then it should be possible to locate a cold region which would be close enough to the pier to be carried in and out from the pier by tidal migrations. Since such migrations are about three miles, a cold region should exist within a few miles of the pier area. In an attempt to locate such a region, a series of temperature observations were made in the summer of 1947 with surface bucket thermometers and BTs.

It was hoped that the cold areas could be located by use of surface temperature data alone, but this did not turn out to be the case, since in this particular area the mixing appeared to take place largely just beneath the surface. However, it was found that BT observations in certain regions were markedly different from others in adjacent regions (see Fig. 3), and that these differences would lead to the expected local temperature changes during tidal migrations. This variation in structure may be explained by considering the variation in maximum tidal current velocity with position, shown also in Fig. 3.

It was possible to make a series of observations showing the relation of cold dip to currents.

For a short time in 1947 a thermograph was again installed at the end of Scripps pier. By observing the variations indicated by this instrument and by considering the nature of the fluctuations shown by past records, it was possible to predict that a cold dip would occur about noon on July 25. The dip occurred as expected. Observations of it were made by B. K. Couper with a 40-foot BT and a bucket thermometer. Currents were determined by the movement of wooden chips thrown onto the water. The dip persisted from 1324 until 1557 PST, during which time the currents were toward the southeast and south. High tide occurred at 1525. At 1625 the currents had begun to shift and the temperature had begun to change. At 1725 relatively isothermal warm water was observed and the currents were toward the east and east-northeast.

To ascertain the temperature-depth structure over a 24-hour period, a series of BT observations was taken from the Scripps pier at half-hour intervals. From these it is possible to determine the temperature variation at any depth (see Fig. 7).

Variations were plotted for the depths 2 and 16 feet above bottom and it was found that the resulting curves were typical of many obtained on the thermographs. It was apparent that large dips did

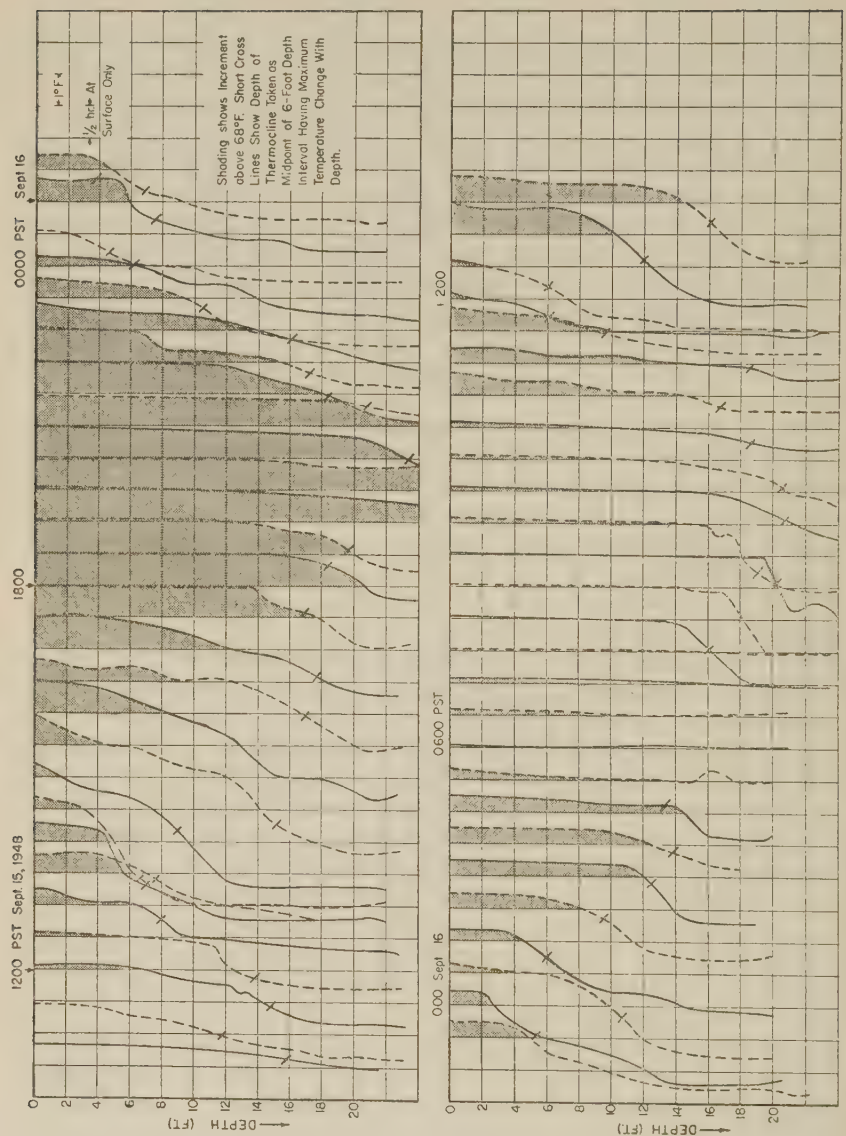


Figure 7. Half-hourly bathythermograms at the end of Scripps Pier.

occur, that these were of approximately tidal period (except for an irregularity at 0730 on September 16 at the bottom), that the smaller dips and those of shorter duration occurred at the surface and at the upper level, and that the ranges are approximately of the correct magnitude for comparison with the average for September shown in Fig. 6. Thus, comparison showed that these BT observations indicate the structural changes which occurred on other days when temperature dips were indicated at two fixed depths by thermographs only.

On the same day as the BT observations used in Fig. 7 were made, Dr. Donald Pritchard[2] arranged to have two ships make similar observations at 18 miles WNW and NW of the Scripps pier. Variations in depth of the thermocline at these positions and at the Scripps pier were determined and plotted. From these data three conclusions could be drawn. First, internal waves having a period of approximately 12 hours occurred at all three locations; second, the range of vertical motion was as great as 40 feet; third, the thermocline was found at a greater depth offshore than at the pier.

Further information on the nature of internal waves near shore was obtained from BTs obtained by a vessel which went out and returned along the same path, repeating observations in the same locations on both legs of the cruise. The temperatures at given longitudes changed markedly between outgoing and return trips.

In August 1948 a cruise to Lower California observed the width of certain low-temperature areas which had been found by Dr. Carl Hubbs along the coastline. In one observed area close to the coast the temperature averaged 58.4° at four stations and in another area 57.2° at six stations, while all of the temperatures seven miles or more offshore, except one, were above 64° F. The width of these near-shore low temperature bands was approximately two miles, and progressing seaward, a transition band of about five miles was measured before the warmer offshore water was reached. From shore seaward, the isotherms dropped so rapidly that the water inshore was many degrees colder than that observed at any depth down to 45 feet at the seaward end of the BT sections across these cold regions. In one section, the inshore stations were somewhat warmer than those a short distance offshore, indicating that the major cooling effect had not occurred at the immediate coastline in this locality. Comparing pier temperatures with values some 15 miles offshore, it is apparent that the maxima of surface temperatures at the pier from April through August occur at times when the water from the coast outward is of uniform or nearly uniform temperature.

[2] Then at the U. S. Navy Electronics Laboratory at San Diego, California.

On the the other hand, when a dip occurs at the pier, the inshore water seems to be much colder than that offshore. This supports the conclusion that offshore changes are more gradual and less marked than temperature changes within one or two miles of the coast and it indicates further the presence of cold dips near shore.

CONCLUSION

The erratic nature of certain coastal sea temperature records which are based on a few observations at specific times of day are explained by the action of internal waves and by periodic migration of cold spots formed over shallow regions of irregular bottom by horizontal variations in velocity of tidal current in the presence of stratified water layers. Proper understanding of the observed variations greatly increases the value of coastal observations. Such observations should be fully utilized since they may readily be obtained from the shore or from piers and docks.

BIBLIOGRAPHY

ARTHUR, R. S.
1954. Oscillations in sea temperature at Scripps and Oceanside Piers. Deep-Sea Research, *2:* 107–121.

LEIPPER, D. F.
1950. Dissertation for Ph.D. Scripps Institution of Oceanography, University of California. 29 pp.

LAMB, HORACE
1932. Hydrodynamics. 6th Ed. Cambridge University Press. 738 pp.

FLEMING, R. H.
1938. Tides and tidal currents in the Gulf of Panama. J. Mar. Res., *1* (3): 192–206.

SVERDRUP, H. U., M. W. JOHNSON AND R. H. FLEMING
1946. The Oceans: Their Physics, Chemistry, and General Biology. Prentice-Hall, Inc., New York. 1087 pp.

McEWEN, G. F.
1916. Summary and interpretation of the hydrographic observations made by the Scripps Institution for biological research of the University of California, 1908 to 1915. Univ. Calif. Publ. Zool., *15* (3): 255–356.
1938. Some energy relations between the sea surface and the atmosphere. J. Mar. Res., *1* (3): 217–238.

CARBOHYDRATE IN SEA WATER[1]

By

GEORGE J. LEWIS, JR. AND NORRIS W. RAKESTRAW

Scripps Institution of Oceanography
La Jolla, California

ABSTRACT

Substances which respond to analytical tests for carbohydrate (with anthrone or N-ethyl carbazole) were found in Pacific Coast water in quantities of from 0.1–0.4 mg/L. In coastal lagoons, however, concentrations as high as 8 mg/L were observed. Normally, most of this is in a dissolved rather than suspended state.

We have little real information about the nature and amount of dissolved organic matter in the sea. Therefore, when it was reported some time ago by Collier, et al. (1950, 1953) that substances having the general chemical properties of carbohydrates had been detected in the Gulf of Mexico in amounts up to 50 mg/L, we began an investigation to find out whether the same thing occurs in or near our area of the Pacific Coast. The earlier work of Krogh (1934), which has impressed us as one of the most important and reliable contributions to this problem, indicated a fairly constant level of dissolved organic matter, at about 5 mg/L. This conclusion, however, was intended to be applicable to the open sea, and it is entirely possible that local conditions, especially near shore, vary widely from such a norm. Indeed, the recent report of Kay (1954) on the distribution of dissolved organic carbon in the shallow waters of the Kielerbucht shows this to be true, although the maximum which he found was only about twice that which Krogh found in open water. Kay's method of of analysis was a modification of Krogh's.

Following the initial report concerning the Gulf of Mexico, already mentioned, Wangersky (1952) endeavored to identify the substances concerned and reported the isolation of a compound having the general absorption spectrum of dehydro-ascorbic acid; he also reported a substance which "gives some indication of being a rhamnoside" and which was said to be present in inshore waters in concentrations up to 0.1 g/L.

REAGENTS AND METHODS

Anthrone. Dissolve one gram of anthrone reagent[2] (recrystallized from benzene and petroleum ether) in one liter of concentrated sulfuric

[1] Contribution of the Scripps Institution of Oceanography, New Series No. 781.

[2] The reagent used was obtained from the Jasonols Chemical Corporation, 1085 Myrtle Ave., Brooklyn 6, New York.

acid. Store in a dark glass-stoppered bottle and keep refrigerated when not in use. Allow the freshly prepared solution to stand four hours before using. It is stable for at least one week.

N-ethyl Carbazole. Dissolve one gram of recrystallized (from ethanol and water) N-ethyl carbazole in one liter of 90% sulfuric acid which has been cooled in an ice bath. Store the solution in a dark glass-stoppered bottle and keep refrigerated when not in use. Avoid exposure to sunlight and air as much as possible. The reagent is stable for at least two days.

Standard Sucrose Solution. Dissolve 500 mg of reagent-grade sucrose in one liter of doubly distilled water and dilute 10.00 ml of this to one liter for a working standard in the range from 0 to 5 mg/L. Add a few drops of saturated mercuric chloride solution as a preservative and store in a refrigerator.

Apparatus. A 70° C (±0.2) water bath is required to develop color with N-ethyl carbazole. Aminco absorption cells, 100 mm in light path and 16 mm outside diameter, contain about 16 ml and are suitable for the sample volumes involved. Simple adaptors can be made to permit their use in a Beckman DU spectrophotometer. Glass-stoppered Pyrex bottles of 60 ml capacity serve well as reaction vessels.

Anthrone Method. Transfer 7.5 ml of filtered sea water to a 60-ml bottle and slowly add 15.0 ml of anthrone reagent with as little mixing as possible so as to form two discrete layers. Then mix thoroughly and immediately place the stoppered bottle in a pan of water which is maintained near room temperature. Between 15 and 25 minutes later, transfer the sample to a 100-mm absorption cell and read the optical density at 627 mμ. Determine standard curves daily, diluting with doubly distilled water. The range from 0 to 5 mg/L is suitable for a 100-mm cell. Determine reagent blanks with 7.5 ml of doubly distilled water.

N-ethyl Carbazole Method. Measure a 2.5-ml sample of filtered sea water and transfer it to a 60-ml bottle. Add 22.5 ml of N-ethyl carbazole and mix thoroughly. Immediately place the sample in a 70° C (±0.2) water bath and leave for exactly 30 minutes. Transfer the sample bottle from the water bath to a refrigerator for 15 to 20 minutes and then allow it to come to room temperature. Avoid exposure to light at all times. Between 30 to 60 minutes after removal from the water bath, determine the optical density at 562 mμ. Determine standard curves and reagent blanks daily with doubly distilled water.

Discussion. Consistent results with anthrone are largely dependent upon the purity of the reagent and on the careful control of temperature immediately after the reagent and sample are mixed. The purity of the anthrone is essential in maintaining constant calibration curves. If the reagent is not recrystallized, changes as great as 25% may occur in one day; with a recrystallized reagent, less than 5% change was observed in a week. The heat of dilution resulting from mixture of concentrated sulfuric acid with the aqueous sample is sufficiently reproducible to limit the error to less than 5%.

For a given sucrose concentration, the color intensity is 20% greater in sea water of 33 ‰ salinity than in distilled water. This effect is apparently due to the chloride ion. A correction factor must therefore be used when calculating results based on standard curves and reagent blanks determined with distilled water solutions.

In general, the N-ethyl carbazole method is considerably less satisfactory. The reddish-violet reaction product is destroyed easily by sunlight and oxidizing agents, with a resultant dark green coloration. Although there is no salt error with the reagent, the procedure is more laborious and exacting because of the rigid temperature requirements.

The reactions of the reagents with a large number of carbohydrates were studied in some detail. Both have about the same sensitivity with different carbohydrates (within a factor of 2), excepting xylose and arabinose, to which anthrone is much less sensitive. The two behave about equally with sucrose, the standard which was chosen. Further details of the two methods will be reported elsewhere at a later date.

EXPERIMENTAL

Unless otherwise indicated, all samples (to be described later) were filtered through a type-HA "millipore" filter having a porosity of 0.5 μ (Goldberg, et. al., 1952). It was established that passage through these filters does not introduce extraneous carbohydrate. The material in this filtrate will be called "dissolved carbohydrate" with the full realization that it may contain some colloidal forms as well as other interfering substances which may show the same color reactions.

A number of samples of surface sea water were collected from the Scripps Pier and from the kelp beds off La Jolla, California, in May and June 1952 and in August 1954; these were analyzed for dissolved carbohydrate at various times after collection. The methods with both reagents were used on several of these; the results are given in Table I in terms of sucrose concentrations. Each value represents the average of from 2 to 11 determinations made on both untreated aliquots and

TABLE I. Carbohydrates in filtered sea-water samples. Concentrations in mg/L using a sucrose standard.

Sample	Method		Location	Date	
	Anthrone	N-Ethyl Carbazole		Collected	Analyzed
A	—	0.14	Pier	6– 9–52	6–11–52
B	—	0.22	Pier	6–11–52	6–12–52
C	0.38	0.22	Pier	6–17–52	6–18–52
D	0.29	—	Pier	8– 9–54	8– 9–54
D	0.26	—	Pier	8– 9–54	8–10–54
E	0.25	—	Pier	8–10–54	8–10–54
F	0.43 (not filtered)	—	Kelp Beds	5– 1–52	5– 6–52
G	0.30	—	Kelp Beds	4–29–52	5–14–52
G	0.37	—	Kelp Beds	4–29–52	5–18–52
G	0.22	—	Kelp Beds	4–29–52	5–21–52
H	0.30	0.11	Kelp Beds	6–18–52	6–19–52
H	0.21	0.15	Kelp Beds	6–18–52	6–20–52
H	0.45	0.38	Kelp Beds	6–18–52	6–24–52
I	—	0.35	Kelp Beds	6–18–52	6–19–52
I	0.16	0.11	Kelp Beds	6–18–52	6–20–52
I	0.20	0.25	Kelp Beds	6–18–52	6–24–52
Average	0.30	0.21			
Std. Dev.	0.09	0.08			

on portions which were evaporated to concentrate the carbohydrate material. Standard deviations within these groups of multiple analyses were 0.09 and 0.08 mg/L for anthrone and N-ethyl carbazole, respectively. Subsequently the anthrone reagent, because of its greater convenience, was used for most but not all determinations.

Table II shows carbohydrate content as a function of depth for four shallow-water stations at half-mile intervals off Scripps Pier; these samples, taken two days after the peak of a heavy phytoplankton bloom, were not filtered, and the values therefore include any

TABLE II. Carbohydrate content at shallow inshore stations off Scripps Pier on Sept. 3, 1952, following a heavy phytoplankton bloom. Concentrations in mg/L in terms of a sucrose standard. Unfiltered samples.

Depth	Station			
(m)	1	2	3	4
0	0.36	0.26	0.32	0.26
3	0.31	0.26	0.29	0.29
7	0.24			
10		0.31	0.27	0.19
20		0.24	0.22	0.15

Standard deviation of duplicate analyses of each sample : 0.06.

particulate matter hydrolyzed upon addition of anthrone reagent in concentrated sulfuric acid. Duplicate analyses were made on all samples, and the standard deviation between duplicates was 0.06 mg/L.

A number of unfiltered samples, taken from various depths in the general area 1000 to 2000 miles off the coasts of Central America and Mexico were analyzed by the anthrone method; similarly, samples from 100 to 200 miles off the coast of southern California were analyzed with N-ethyl carbazole. These results, not tabulated in detail, fell within the range below 0.7 mg/L of dissolved carbohydrate, with a slight tendency to diminution with depth.

TABLE III. Carbohydrate in coastal lagoons of the San Diego area. Concentrations in mg/L in terms of a sucrose standard.

Lagoon	Carbohydrate	Chlorinity (°/₀₀)
San Luis Rey	3.9	12.2
Loma Alta Creek	3.4	16.0
Buena Vista (Upper)	2.9	4.0
Buena Vista (Lower)	2.2	4.7
Encinas	0.3	18.7
San Marcos	3.4	26.8
San Elijo	5.3	22.2
Del Mar	7.9	3.4
Sorrento (Upper)	1.6	19.7
Sorrento (Lower)	0.5	19.3
San Miguel	0.3–0.9	—

Determinations with the anthrone reagent were made on filtered samples from nine coastal lagoons in the vicinity of San Diego. Results are given in Table III together with chlorinity values as an indication of the influence of fresh-water drainage, sea-water flushing during the tidal cycle, and evaporation. Encinas, Sorrento, and San Miguel lagoons are open at the mouth, thus permitting various degrees of flushing during the tidal cycle; the six other lagoons are separated from the ocean by sand bars or cobble berms. In San Miguel lagoon the indicated values gradually increased from 0.3 mg/L at the mouth to 0.9 at the upper end of an arm 300 yards inland. This lagoon was sufficiently shallow to be completely drained with each low tide. In general, there was a direct correlation between the carbohydrate content and the occurrence of visible suspended organic matter in each lagoon.

When appraising the results reported here, note that the lower end of the sensitivity range of both analytical reagents was used in most cases. The reproducibility of results, as indicated by the standard deviation among multiple analyses of the same sample, is slightly less

than 0.1 mg/L. Indicated concentrations in many of the samples were not more than three or four times this amount, thus making any conclusions dubious. The mere absence or presence of two or more different carbohydrate constituents or of other substances to which the reagents may be sensitive could produce the differences observed in such cases. However, it seems definitely established that, in the open sea at least, there is in our part of the Pacific Ocean no such concentration of "carbohydrate" as has been reported in the Gulf of Mexico.

On the other hand, a small amount of such material does seem to be present in our closed lagoons as a result of land drainage or other causes, but this amount is not more than one-tenth of that in the Gulf region. And, if we may assume that particulate carbohydrate material is readily hydrolyzed by strong acids, the similarity of results from filtered and unfiltered samples indicates that the relative amount of such particulate material is small.

LITERATURE CITED

Collier, A.
1953. On the significance of organic compounds in sea water. Trans. 18th Amer. Wildl. Conf., pp. 463–468.

Collier, A., S. Ray and W. Magnitzky
1950. A preliminary note on naturally occurring organic substances in sea water affecting the feeding of oysters. Science, *111:* 2876.

Goldberg, E. D., M. Baker and D. L. Fox
1952. Microfiltration in oceanographic research. I. Marine sampling with the molecular filter. J. Mar. Res., *11:* 194–204.

Kay, H.
1954. Untersuchungen zur Menge und Verteilung der organischen Substanz im Meerwasser. Kieler Meeresforsch., *10:* 202–214.

Krogh, A.
1934. Conditions of life at great depths in the ocean. Ecol. Monographs, *4:* 430–439.

Wangersky, P. J.
1952. Isolation of ascorbic acid and rhamnosides from sea water. Science, *115:* 658.

NOTE ON WIRE-ANGLE IN OCEANOGRAPHY

By

HÅKON MOSBY

Geophysical Institute, University of Bergen

Recently Watson[1] made known an interesting experiment performed at the David Taylor Model Basin of the U. S. Navy. His experiment showed that a cable inclined to a horizontal stream experiences mainly a force which is normal to the cable. This agrees with experiments performed in the laboratory of the Oceanographic Institute, Gothenburg.[2]

In my paper,[3] the basic equations were established by assuming that the stress is horizontal and equal to that on the vertical projection of the cable. According to the experiments of Watson and Kullenberg my assumption does not hold and therefore the accuracy of the derived corrections of depth must be questioned.

The component of force acting at right angles to the wire element is equal in both cases, but in my assumption of 1951 an additional component acting along the direction of the wire was introduced. This leads to an expression of the angle between the wire and the horizontal (see Mosby, 1952: 4, formula 1) which can readily be integrated. On Watson's assumption the corresponding expression becomes

$$\tan \alpha = \frac{(m + s)\ p - \eta\, f\, v^2 \int_0^s \sin^2 \alpha \cos \alpha\, ds}{\eta\, f\, v^2\, (\eta + \int_0^s \sin^3 \alpha\, ds)},$$

which is not easy to handle. However, there is no difficulty in performing numerical integration in both cases. This has been done for a wire 1000 m long towed at 50 and 100 cm/sec respectively (examples illustrated in Mosby, 1952: fig. 7). It was found that the lead and

[1] Watson, E. E. 1953. An experiment to determine the hydrodynamic forces on a cable inclined to the direction of flow. Mar. Res., *12* (3): 245–248.

[2] Kullenberg, B. 1952. Sur la résistance à l'avancement d'un cylindre dans une eau courante si le cylindre forme un angle aigu avec la direction du courant. Cah. Cen. Recher. Ét. Océanogr., No. 5.

[3] Mosby, H. 1952. Wire-angle in Oceanography. Årb. Univ. Bergen, Naturv. Rekke Nr. 2: 1–26.

the lower end of the cable would reach greater depths according to my assumption when the same value was used in both cases for the resistance coefficient $\eta = 0.00087$. In the case of Watson's assumption, if this value is reduced by about 7% (or if the speed of towing is reduced by about 3%), then his and my assumptions lead to nearly identical results. For practical purposes corrections of depth will usually be determined from the wire angle at the surface; such corrections will then be practically independent of the basic assumption.

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PREFACE

The Laboratory of Oceanography, established by the Office of Naval Research at Woods Hole, Massachusetts, was dedicated on June 21, 1954. The event marked a milestone in the history of oceanography and provided tangible recognition of the value of this science to the nation. Just 25 years had elapsed since a committee of the National Academy of Sciences submitted a report that stressed the importance of oceanography. This report eventually led, through private benefactions, to the establishment of the Woods Hole Oceanographic Institution and the Laboratory of Oceanography at the University of Washington and to substantial gifts to the Scripps Institution of Oceanography and the Bermuda Biological Station. Without this impetus it is doubtful whether American oceanography would have gained sufficient stature to justify the investment by the nation in the new Laboratory.

To celebrate the dedication of the Laboratory of Oceanography, the National Academy of Sciences-National Research Council arranged for a Convocation to present and discuss the problems of the science of the sea in their broadest sense. The result was an assemblage of men of eminence and talent such as never before had graced an oceanographic gathering in this country.

The present volume is a record of the addresses and scientific papers delivered at the oceanographic convocation. Included also are the contributions of two authors, Dr. Walter H. Munk and Dr. Ph. H. Kuenen, who were unexpectedly prevented from attending.

The Convocation was sponsored jointly by the National Academy of Sciences-National Research Council, the Office of Naval Research, and the three scientific institutions in Woods Hole; namely, the Marine Biological Laboratory, the Woods Hole Oceanographic Institution, and the Fish and Wildlife Service. The meetings were held in the auditorium of the Marine Biological Laboratory. Rear Admiral Edward H. Smith, USCG (Ret.), Director of the Woods Hole Oceanographic Institution, served as chairman at the opening session. Chairmen for the sessions on Physical Oceanography were Dr. H. U. Sverdrup of the Norsk Polarinstitutt and Captain J. B. Cochran, USN, Hydrographer, U. S. Navy; for the sessions on Biological Oceanography, Professor Trygve Braarud, Universitetets Biologiske Laboratorium, Oslo and Dr. Henry B. Bigelow of Harvard University; and for the sessions on Geology and Geophysics and on Meteorology, Dr.

W. W. Rubey of the U. S. Geological Survey and Chairman of the National Research Council and Dean Athelstan F. Spilhaus of the Institute of Technology, University of Minnesota.

The Scientific Program was arranged by a committee consisting of Drs. Detlev W. Bronk, Chairman, Philip B. Armstrong, Henry B. Bigelow, Carl Eckart, Richard H. Fleming, G. Evelyn Hutchinson, Bostwick H. Ketchum, Daniel Merriman, Donald W. Pritchard, Lionel A. Walford, H. R. Gault, and Mr. Gordon G. Lill.

Alfred C. Redfield
Editor

Woods Hole, Mass.
July 30, 1954

~ *GENERAL ADDRESSES*